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CATECHOLAMINES AND GASTRIC ACID SECRETION

A clinical and experimental study



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by

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The present thesis is based on the following papers, which will be referred to in the text by their Roman numerals

- I Effects of food on plasma catecholamine and gastrin levels in patients with duodenal ulcer and normal volunteers.
U. Angerås, L.-O. Farnebo, H. Graffner, B. Hamberger, K. Uvnäs-Moberg and J. Järhult.
Digestion 25:205-210, 1982.
- II Elevated plasma levels of noradrenaline in duodenal ulcer.
J. Järhult, U. Angerås, L.-O. Farnebo, H. Graffner and B. Hamberger.
World J. Surg. 7:385-389, 1983.
- III The role of catecholamines in the control of gastric acid secretion during insulin hypoglycaemia and modified sham feeding.
H. Graffner, U. Angerås, L.-O. Farnebo, B. Hamberger, J. Oscarson and J. Järhult.
Acta Chir. Scand. 149:389-392, 1983.
- IV Is there a relationship between gastric acid secretion and plasma catecholamines in duodenal ulcer disease?
H. Graffner, L.-O. Farnebo, B. Hamberger and J. Järhult.
Digestion, in press.
- V The effect of β -blockade on gastric acid secretion, gastrin release and plasma catecholamine concentrations during modified sham feeding in duodenal ulcer disease.
H. Graffner and J. Järhult.
Scand. J. Gastroent, in press.
- VI Pirenzepine depresses plasma noradrenaline in duodenal ulcer patients.
H. Graffner, L.-O. Farnebo, J. Holst and J. Järhult.
Submitted to Life Sci.
- VII Effects of upper abdominal sympathectomy on gastric acid, serum gastrin and catecholamines in the rat gut.
H. Graffner, M. Ekelund, R. Håkanson, J. Oscarson, E. Rosengren and F. Sundler.
Scand. J. Gastroent, in press.
- VIII Peptide-containing nerve fibers in the stomach wall of rat and mouse.
E. Moghimzadeh, M. Ekelund, H. Graffner, R. Håkanson and F. Sundler.
Submitted to Gastroenterology.



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As a part of nature, every animal organism represents a very complicated, closed system, the internal forces of which, at any given moment, as long as it exists as such, are in equilibrium with the external forces of its environment.

Ivan Petrovich Pavlov 1909.

1. INTRODUCTION

The regulation of gastric acid secretion has been studied extensively for many years mainly due to the fact that suppressed acid output promotes peptic ulcer healing. Pavlov's classical studies (1), showing that sham feeding stimulated gastric acid secretion, and the observation that vagotomy abolished this response, stimulated work on the influence of the parasympathetic nervous system on gastric acid output. The discovery of gastrin (2) opened up a new area of research which finally resulted in the isolation and sequencing of the hormone (3). During the 70's, new neurotransmitters - the neuropeptides - were introduced and "the enteric nervous system" now includes 16 or more putative transmitters (4,5).

Stress has for many years been implicated in ulcer pathogenesis. The classical studies by Beaumont (6) and Wolf (7) demonstrated that both mucosal hyperemia and secretion of gastric juice were augmented by anxiety and distress and more recent studies have shown that stressful situations, such as severe emotional trauma, induce an increase in acid secretion (8,9). Whether or not a stressful event precedes the onset of ulcer symptoms as suggested by Davies (10), Alp (11) and Feldman (12) has recently been questioned (13).

Although stress and an increased sympathetic drive might be pathogenetic factors in ulcer diathesis, data concerning the influence of the sympatho-adrenal system (SAS), i.e. of sympathetic nerve activity and circulating catecholamines, on gastric acid secretion are scanty and controversial.

1.1 Anatomy of the sympathetic nervous system with special reference to the innervation of the stomach.

Preganglionic fibers arising from the thoracic and lumbar part of the spinal cord are referred to as the thoracolumbar or "sympathetic" division of the autonomic nervous system. Efferent sympathetic fibers to the gut are carried mainly through the splanchnic nerves (Fig.1). The preganglionic fibers emanate from Th₅₋₉ and contact neurones in the coeliac and superior mesenteric ganglia. Most of the cell bodies in the prevertebral ganglia contain histochemically demonstrable noradrenaline. Noradrenergic fibers, but no cell bodies, are found within the gut wall where they supply the ganglia of the myenteric plexus, and also ramify among the neurones of the submucous ganglia or form plexus around arteries. A few fibers extend into the mucosa and form an open network around the glandular base. Some noradrenergic

fibers arise in the stellate and the superior cervical sympathetic ganglia and follow the vagal nerves to the stomach (14, 15). For a review of the adrenergic innervation of the gastrointestinal tract see Furness (16, 17).

The adrenal medulla constitutes a part of the sympathetic nervous system and is in effect a modified sympathetic ganglion in which the postganglionic neurones have lost their axons and become secretory cells. The adrenal medulla secretes both adrenaline and noradrenaline into the blood although the former substance is released in the highest concentrations. Noradrenaline is also released from terminals of postganglionic sympathetic neurones and deposited directly at the target cells. Noradrenaline in plasma, both at rest and in response to various stimuli, emanate from these nerve terminals (18). The plasma catecholamines are transported to almost all cells in the body and they have been ascribed a variety of cardiovascular and metabolic effects. Both adrenaline and noradrenaline have a short half-life in circulation (less than 2 min) and are metabolized to biologically inactive products by oxidation and methylation or are taken up by the postganglionic sympathetic neurones.

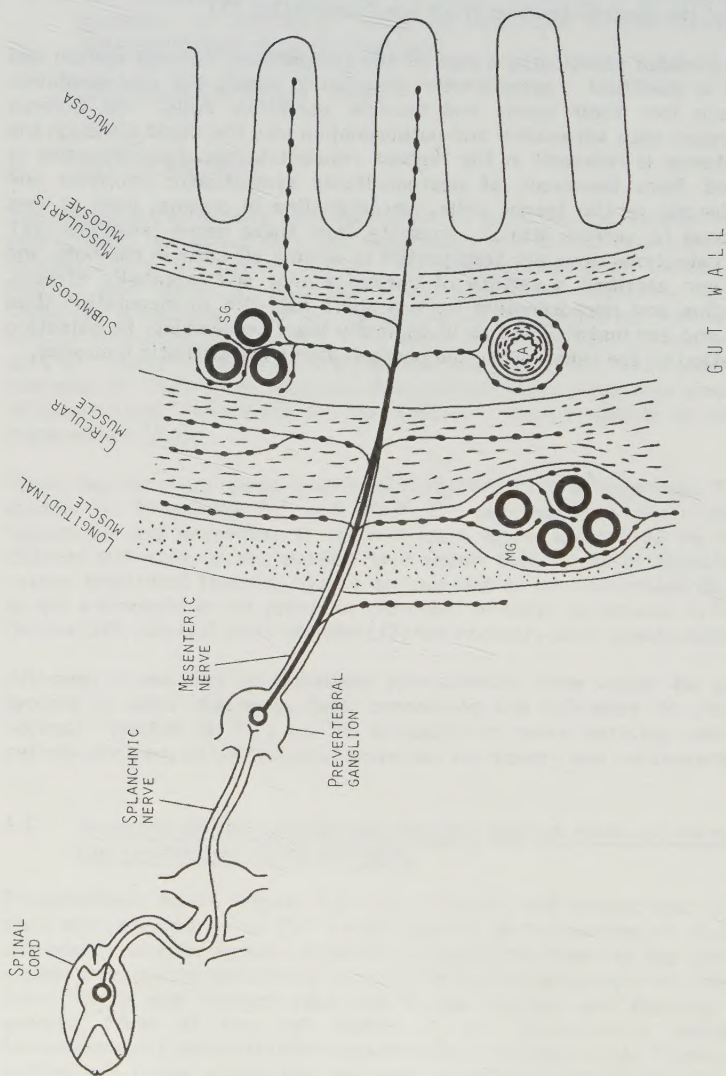


Fig 1. Schematic drawing of the efferent sympathetic pathway to the gut. SG=submucous ganglia. MG=myenteric ganglia. A=artery

1.2 Previous studies

Pavlov (19) was the first to postulate the existence of fibers from the sympathetic chain to the stomach. Section of the splanchnic nerves inhibited the increase in gastric secretory volume induced by sham feeding. Bickel (20) suggested that the sympathetic branches stimulated secretion of organic material and enzymes while the vagal nerves were responsible for the liquid portion of the gastric juice. Electrical stimulation of the splanchnic nerves was found to increase gastric secretion (21) but on the other hand Moll and Flint (22) reported in 1928 that section of the splanchnic nerves in dogs resulted in an increase in the free and total acidity of gastric juice. They also showed that injection of adrenaline induced "a striking lowering of the HCl secretion." Splanchnicectomy produced peptic ulcers in dogs and rabbits (23, 24) and this was confirmed in man (25). With the development of methods for measurement of tissue blood flow, studies using splanchnic nerve stimulation showed a correlation between acid secretion and mucosal blood flow (26, 27).

When specific and highly effective sympathomimetics and sympatholytics became available a renewed interest in the sympathetic nervous control of gastric acid secretion was noted (28-31). However, the results of these studies must be interpreted with caution since the various pharmacological compounds may affect the SAS in general.

The insulin test (32) has for a long time been used for testing vagal function. Due to a possible influence of the SAS on the resulting acid secretion induced by hypoglycaemia (33) the modified sham feeding test (MSF) has been claimed to be superior to the insulin test (34) in acid secretory tests after vagotomy. However, no studies have been performed to establish if the SAS has any influence on MSF.

Measurement of plasma catecholamines has earlier been difficult to perform (35) but the development of new techniques for estimation of plasma catecholamines became available during the late 70's (36, 37). Thus, a renewed interest in the influence of the SAS in health and disease was noted (38). Reports of the influence of circulating catecholamines on duodenal ulcer (DU) disease are few (39-42). However, these studies show that DU patients have increased levels of noradrenaline in plasma (39) and that DU patients respond with a greater increase in serum gastrin than healthy volunteers to infusion of adrenaline (42). Vagotomy - one of the corner stones in ulcer therapy - did not induce any changes in circulating catecholamine levels (40). With the development of new therapeutic tools for ulcer healing and prevention of ulcer relapse - histamine₂-receptor blockers (43) and selective antimuscarinic compounds (44) - further studies of the role of plasma catecholamines on acid secretion and pharmacologically induced acid depression will be possible and might further establish the role of circulating plasma catecholamines in DU disease.

The controversial results of earlier studies using surgical extirpation of sympathetic ganglia might be due to the fact that there was no way of assessing the completeness of denervation. With the development of histochemical methods for visualization of adrenergic nerve fibers (45) a semi-quantitative method became available. New methods for measurement of tissue catecholamines (46) have been published during the last decade but has hitherto not been used to assess the degree of denervation.

1.3 Aim of the present investigation

The aim of the present investigation was to study the role of catecholamines with respect to gastric functions

in man:

1. in response to food (I)
2. before and after vagotomy (II+IV)
3. during stimulation with insulin and modified sham feeding (III)
4. in response to histamine (H_2) - receptor blockade (IV)
5. during active ulceration (IV)
6. during β -blockade and stimulation with modified sham feeding (V)
7. in response to a selective antimuscarinic compound (VI)

after upper abdominal sympathectomy in rats with special reference to:

8. tissue concentrations of catecholamines (VII)
9. gastric acid secretion (VII)
10. peptidergic innervation in the stomach (VIII)

2. METHODS

2.1 Human studies

2.1.1 Material

A total of 46 patients (median age 55 years, range 33-68 years) was studied. 6 patients participated in more than one study. Criteria for inclusion were a history of peptic ulcer for more than 1 year and the presence of a duodenal ulcer (DU) verified by endoscopy. During the periods of study the patients were without medication (exceptions in paper IV, V) and without symptoms of active ulcer (exception in paper IV). Six volunteers (median age 33 years, range 30-37 years) participated in the study described in paper I.

2.1.2 Experimental protocol

The details of the different methods used are given in the respective papers and only a short summary is given here. Acid secretory tests were performed using a nasogastric tube connected to a pump for intermittent suction. Stimulation was performed using pentagastrin (Peptavlon^R-ICI 6 µg/kg BW s.c.), insulin (Insulin Novo Actrapid^R 0.1 IU/kg BW i.v.) or food consisting of shredded sirloin steak, potatoes, vegetables and a glass of milk. The meal contained 39 g protein, 30 g fat and 30 g carbohydrate with an energy content of 2.32 kJ. In the modified sham feeding tests, milk was replaced by water and each subject was instructed to chew the food and then spit it out.

β-Blockade, using propranolol (Inderal^R ICI-Pharma 100 mg) orally 2 hours before start of testing, was performed in paper V. In paper IV cimetidine (Tagamet^R SKF 200 mg t.i.d and 400 mg at night) was administered orally to a group of patients. Plasma samples were taken before and on the last two days of treatment. Also, in paper IV a group of patients with active DU were treated with ranitidine (Zantac^R Glaxo 150 mg b.i.d for 4 weeks). Plasma samples were taken before start of treatment and after healing had occurred and treatment stopped. Highly selective vagotomy (HSV) was performed on patients described in papers II-IV. The surgical technique was essentially that of Amdrup (47) and Johnston (48). In paper VI pirenzepine (Boehringer Ingelheim 10 mg) was injected intravenously after a 30 min rest.

All studies were performed after an overnight fast and tobacco abstinence. Blood sampling was performed through a cannula implanted in an antecubital vein. The first sample for analysis of plasma catecholamine levels was taken after a 30 min rest (in acid secretory tests 30 min after the introduction of a nasogastric tube) in a comfortable armchair. Basal samples were always taken twice throughout the experiments. Blood samples were taken at intervals during stimulation as described in the different studies.

2.1.3 Biochemical analyses

Gastric juice was sampled during 15 min periods. The volume of the gastric juice was recorded and a 5 ml aliquot titrated to pH 7 using 0.1N NaOH in an automatic titrator (Radiometer Copenhagen). Basal acid output (BAO) was the acid secretion one hour prior to stimulation and peak acid output (PAO) the two highest 15 min periods multiplied by two.

Blood samples were stored on ice in heparinized tubes and cold centrifugation was performed less than one hour after sampling. Plasma samples were stored at -70° C (group 3 in paper IV; -20°C).

Measurements of plasma catecholamine levels were identical in all studies. Plasma catecholamines (adrenaline, dopamine and noradrenaline) were analyzed with high pressure liquid chromatography according to Hallman et al (36). In our hands this method is very sensitive and permits determination of concentrations down to 0.1 nM. The intra and interassay coefficients are around 2,5 %.

Serum gastrin concentrations were determined in three different laboratories using radioimmunoassay and antiserum 2604-8 from Rehfeld's laboratory (49). Striking differences in serum gastrin concentrations between the laboratories were noted. However, in our studies comparison of gastrin measurements are made only in paired experiments using values from a single laboratory and no attempt has been made to compare serum gastrin values from the three different laboratories. Somatostatin analyses were determined with radioimmunoassay as previously described (50).

2.2 Rat experiments

2.2.1 Material and experimental protocol

A total of 120 male Sprague Dawley rats weighing approximately 200 g were used in the experiments. They were fed a standard diet of food pellets and tap water. Prior to surgery or studies of gastric acid secretion the rats were kept without food but with free access to water in cages with wire mesh bottoms. The fasting period was 24 h prior to surgery and gastric fistula experiments and 48 hours prior to pylorus ligation and determination of histidine decarboxylase, serum gastrin and tissue catecholamines.

Surgery was performed under clean, but not sterile conditions, using chloralose (sympathectomy) or ether (fistula implantation, vagotomy and pylorus ligation) anaesthesia. Bilateral vagotomy was carried out according to Shay et al (51). Pyloroplasty was performed at the same time. Sympathectomy was performed using an operation microscope and the procedure was essentially that of Holmes (52). Gastric fistula rats were prepared as described by Bel et al (53) using plastic fistulas. Operated rats were left for at least four weeks before sacrifice after a 48 h fast with free access to water. During ether anaesthesia

the animals were bled to death by collecting blood from the aorta. The gut was divided in portions, as described in paper VII, for determination of tissue concentrations of adrenaline, noradrenaline and dopamine. In experiments with gastric fistula rats conscious animals were restrained in Bollman type cages after the administration of 10 ml 0.9 % saline s.c. and after thorough rinsing of the stomach. After one hour of free flow basal acid secretion was collected in two one-hour portions. The gastric juice was collected for 4 further hours after the administration of pentagastrin (Peptavlon^R ICI) 250 µg/kg s.c. or histamine dihydrochloride 25 mg/kg s.c. Pylorus ligation was performed by applying a tight ligature around the pylorus in the manner described by Shay et al (54). At the end of the 4 hour test period the animals were again anaesthetized, the abdomen opened and the entire stomach removed after clamping the oesophagus. The stomach was opened and rinsed with 1 ml of re-distilled water. Stomach contents was sampled in test tubes which were centrifuged. Acid output was determined by titration with 0.02 M NaOH and phenolphthalein as indicator. The results were expressed as µEqv H⁺ per hour.

2.2.2 Biochemical analyses

Tissue samples were weighed and placed in perchloric acid, homogenized and filtered. Tissue catecholamine concentrations were determined by high performance liquid chromatography (46). Histidine decarboxylase activity was determined radiometrically (55) and serum gastrin by radioimmunoassay (49).

2.2.3 Immunocytochemistry

The details of the methods used are given in paper VIII and, since only a small part of that study is concerned with changes after sympathectomy, only a short summary of the methods is given here.

Specimens from the oxyntic gland area and the pyloric gland area were taken. Sections and whole mounts were processed for the immunocytochemical demonstration of gastrin/cholecystokinin (CCK), gastrin-releasing peptide (GRP), vasoactive intestinal peptide (VIP), substance P (SP), leu/met-enkephalin and somatostatin. Pancreatic polypeptide (PP) and neuropeptide Y (NPY) were demonstrated using an antiserum raised against porcine NPY and an antiserum raised against avian pancreatic polypeptide (APP) (56); this antiserum cross-reacts with NPY (57). The immunoreactive material demonstrated by these two antisera in neuronal elements is referred to as NPY. The site of the antigen-antibody reaction was visualized by the indirect immunofluorescence method of Coons et al (58) or by the peroxidase-antiperoxidase (PAP) procedure of Sternberger (59).

2.3 Statistics

In all experiments nonparametric tests (Wilcoxon's signed ranks sum test for paired data and the Mann-Whitney U-test for unpaired data) were used to test significant differences between various variables. Data are, unless otherwise stated, expressed as median and interquartile range.

2.4 Comments to methodology

2.4.1 Catecholamine measurements

Sampling of plasma for catecholamine analysis must be performed under strictly standardized conditions in order to obtain reproducible results. The act of venepuncture itself elevates plasma adrenaline levels significantly (60) and during serial plasma catecholamine estimations an indwelling catheter should be used (61), allowing a 20 min period of rest before the start of sampling (61). Catecholamines which enter the blood are rapidly degraded and/or metabolized and therefore samples must be frozen immediately. After a 30 min storage in room temperature plasma catecholamine content decrease by 30 % (60), whereas no decrease was found during a one hour storage at +4°C when using heparinized tubes (61). Decay in total plasma catecholamine content has also been reported following storage at -20°C (60).

Changes of posture strikingly affect circulating catecholamine concentrations. Thus, Vendsalu (35) found adrenaline and noradrenaline values of 0.04 and 0.36 µg/l in the recumbent man whereas after a 10 min head-up tilt the corresponding values had increased to 0.11 and 0.62 µg/l, respectively. Venous catecholamine content also varies in different parts of the body although there seems to be no differences between the antecubital and superior caval vein (60). In our studies every effort was made to standardize sampling, storage and analyses.

Age has been reported to influence basal catecholamine levels. Pedersen and Christensen (38) found a significant correlation between plasma noradrenaline and age in normotensive volunteers as well as in hypertensive patients. This was confirmed in normotensive volunteers by Lake (61), Esler (62) and Saar (63) as opposed to de Champlain (64) and Cryer (65) who found no correlation between age and noradrenaline. In another study by Christensen et al (39) plasma noradrenaline increased with age in a group of patients with DU but not in a group of healthy volunteers. The above mentioned studies were performed using radioenzymatic methods for determination of noradrenaline. However, using high pressure liquid chromatography with electrochemical detection, Hjendahl (66) could not find any correlation between plasma noradrenaline levels and age in healthy subjects or hypertensive patients. In our study, using essentially the same technique as Hjendahl (37), no correlation was found. The explanation for the difference between the results obtained by the two methods is obscure.

There are essentially three different techniques for analysis of plasma catecholamines; fluorometric, radio-enzymatic and high pressure liquid chromatography with electrochemical detection. With fluorometric methods large sample volumes must be used and radioenzymatic methods are time consuming and expensive. High pressure liquid chromatography with electrochemical detection permits simultaneous determination of noradrenaline, adrenaline and dopamine in concentrations down to 0.1 nmol/l

in less than 1 ml plasma samples. Tissue estimations of catecholamines using the method described by Eriksson and Persson (46) is highly sensitive, providing sampling and storage are standardized. In our studies in rats, specimens were collected during ether anaesthesia and death induced by collecting blood from the aorta. Ether anaesthesia is induced quickly and exsanguination takes less than 30 sec. During prolonged barbiturate anaesthesia (4 h) and haemorrhagic shock plasma adrenaline and noradrenaline levels are found to be lower than in unanaesthetized rats and changes also occur in tissue catecholamine levels (67). Therefore standardized conditions are of utmost importance and paired experiments should always be performed.

2.4.2 Statistics

In studies of biological parameters there is not always a Gaussian distribution of the data. Therefore values should be given as median and range or median and interquartile range. For practical reasons results are often given as mean $\pm$ S.E.M. (paper I-III, VII) but caution must be exercised when interpreting such values, especially the S.E.M. Since Gaussian distribution cannot be assumed, nonparametric tests should be used - Wilcoxon's signed pair's ranks sum test for paired data and the Mann-Whitney U-test for unpaired data are appropriate (68) and have been used in these studies. During studies with humans the desired number of patients or volunteers is often difficult to obtain. However, if a significance level is found, the observation is true and may be regarded as safe but when using small number of observations, it is possible that a true difference will not be found and therefore a false-negative error (type 2 or β -error) may occur.

3. RESULTS

3.1 Circulating catecholamines and acid secretion.

3.1.1 Background

Brandsborg et al (39) reported in 1978 that patients with DU disease had higher plasma levels of noradrenaline than a healthy control group, whereas no differences in adrenaline levels were observed.

The effects of infusion of adrenaline or noradrenaline have been repeatedly studied both in man and laboratory animals and a survey of data available in the literature is shown in Table I and II. In general, noradrenaline seems to depress both basal and stimulated acid secretion whereas the results of adrenaline infusion vary in different studies. Similarly, stimulation of the splanchnic nerves in anesthetized cats causes a reduction in both basal and meal-stimulated acid secretion (26,27). Whether or not the depressant effect of noradrenaline on gastric acid secretion is due to vasoconstriction has not been definitively settled (69,70).

According to some reports insulin-induced acid secretion is suppressed by β -blockade (82,83). Insulin hypoglycemia is also known to release adrenaline and noradrenaline which in turn might induce changes in acid secretion (33,41,77,84). To our knowledge no data are available on the plasma catecholamine concentrations after other types of acid stimulation, such as modified sham feeding (MSF) which represents an alternative way to study vagal activation (34). The studies in paper I-IV were performed in an attempt to further evaluate the possible relationship between circulating catecholamines and acid secretion during basal as well as stimulated (food, insulin and MSF) conditions.

TABLE I

Effects of noradrenaline infusion on acid secretion

AUTHOR	SPECIES	DOSAGE	BAO	PAO-PENTAGASTRIN	PAO-HISTAMINE	PAO-BETANECHOL
Harries 1956(71)	Dog	0.3-1.2 $\mu\text{g/kg/min}$	NT	NT	→	NT
Leonsins 1958(72)	Man	0.5 $\mu\text{g/kg/min}$	NS	NT	→	NT
Pradhan 1962(73)	Dog	75 $\mu\text{g/kg}$	NT	NT	→	→
Cumming 1963(74)	Dog	1-8 $\mu\text{g/kg/min}$	NT	NT	→	NT
Haig 1968(75)	Dog	1 $\mu\text{g/kg/min}$	NT	→	→	→
Jacobson 1970(76)	Dog	0.1-1 $\mu\text{g/kg/min}$	NT	→	→	NT
Christensen 1976(77)	Man	5 ng/kg/min	NS	NT	NT	NT
Christensen 1976(77)	Man	50 ng/kg/min	→	NT	NT	NT
Tani 1979(78)	Rat	25-100 $\mu\text{g/kg}$	NS	→	→	→
Semb 1982(79)	Cat	1 $\mu\text{g/kg/min}$	NT	→	NT	NT
Ekelund 1983 (80)	Rat	50-500 $\mu\text{g/kg/h}$	→	NT	NT	NT

NT = not tested

NS = not significant

TABLE II

Effects of adrenaline infusion on acid secretion

AUTHOR	SPECIES	DOSAGE	BAQ	PAO-PENTAGASTRIN	PAO-HISTAMINE	PAO-BETANECHOL
Pradhan 1962(73)	Dog	40-100 µg/kg	NT	NT	↓	↓
Cumming 1963(74)	Dog	1-8 µg/kg/min	NT	NT	NS	NT
Haig 1968(75)	Dog	1 µg/kg/min	NT	↓	↑	↑
Stadil 1973(33)	Man	25-75 ng/kg/min	↑	NT	NT	NT
Christensen 1976(77)	Man	5 ng/kg/min	↑	NT	NT	NT
Christensen 1976(77)	Man	50 ng/kg/min	NS	NT	NT	NT
Brandsborg 1977(81)	Dog	2-8 µg/min	NS	NT	NT	NT
Tani 1979(78)	Rat	12, 5-50 µg/kg	↑	↑	NT	NT
Semb 1982(79)	Cat	1 µg/kg/min	NT	↓	NT	NT
Yokotani 1983(70)	Rat	0.5-2.0 µg/kg/min	NT	NT	NT	↓ (electrical vagal stimulation)

NT = not tested

NS = not significant

3.1.2 Results (I-IV)

Basal noradrenaline concentration was significantly ($p < 0.02$) higher in DU patients (1.53 (0.8-3.1) nM) than in healthy controls (0.85 (0.5-1.9) nM). In contrast basal plasma adrenaline and dopamine concentrations were low and not different in normal volunteers and DU patients. Food induced a significant increment in plasma noradrenaline concentration in both normals and DU patients, whereas the two other catecholamines remained unchanged (Fig 2). After the meal, plasma noradrenaline concentration fell rapidly towards the control level in normal subjects in contrast to DU patients in whom noradrenaline levels remained elevated during the whole period of observation (90 min after the meal).

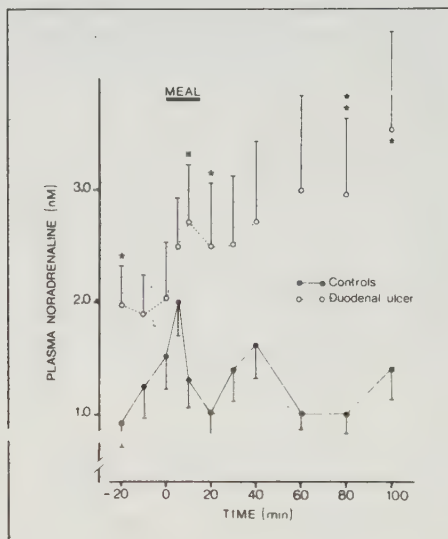


Fig 2. Changes in plasma noradrenaline concentration in response to a standard meal in 8 patients with DU disease and 6 healthy controls. Asterisks indicate a significant difference between the two groups. * $p < 0.05$; ** $p < 0.01$.

Insulin hypoglycemia raised plasma adrenaline and noradrenaline levels whereas MSF did not evoke any significant changes (Fig 3). Yet, acid secretion was equally stimulated during the insulin test and the MSF (Fig.4). In conclusion, no correlations were found between basal or augmented acid secretion and circulating plasma levels of adrenaline, dopamine or noradrenaline in DU patients or normals.

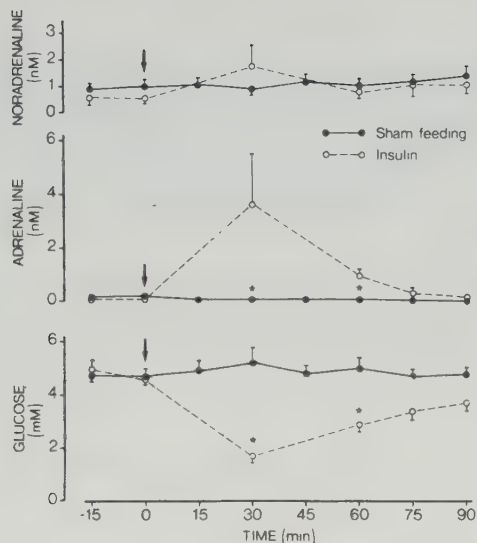


Fig 3. Changes in plasma noradrenaline and adrenaline and in blood glucose concentrations in response to MSF and insulin in 7 patients with DU disease 6 weeks after HSV. Arrows indicate start of stimulation and asterisks indicate significant differences between the two tests (* $p < 0.02$)

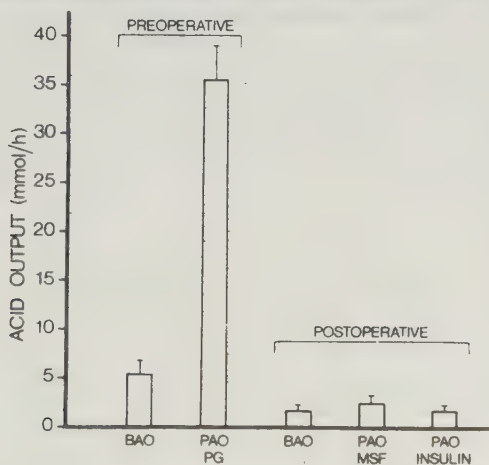


Fig 4. Basal acid output (BAO) and peak acid output (PAO) in response to pentagastrin (PG), modified sham feeding (MSF) or insulin in 7 DU patients before and 6 weeks after HSV. There is a statistically significant difference between BAO pre and postoperatively but not so between the postoperative PAO obtained with insulin or MSF.

3.1.3 Comments

Peptic ulcer is thought to be a psychosomatic disease (85). It has also been suggested that stress, increasing the activity of the SAS, is an etiological factor in ulcer diatheses (6-11) and different kinds of stress has been used to promote ulcer formation in animals (86). Still, little is known about the role of the SAS in peptic ulcer disease and the physiological control of acid secretion and gastrin release.

In 1978 Brandsborg et al (39) reported that DU patients have increased plasma levels of noradrenaline. Our studies (I-IV) confirm this finding and have extended the observation to show that plasma noradrenaline concentrations are also exaggerated in response to food and insulin but not in response to sham feeding. The finding of elevated plasma noradrenaline levels in DU patients was confirmed recently by Bekker et al (87).

Hyprenoradrenalinemia possibly reflects an increased activity in the SAS, in turn caused by stress, compensation for increased vagal activity or alterations in noradrenaline metabolism. The source of the increased circulating noradrenaline concentrations has not been established. As the circulating adrenaline concentrations are normal in DU patients it may be assumed that the noradrenaline emanates from the noradrenergic nerve terminals. If the excess noradrenaline is derived from a specific organ system e.g. the gastrointestinal tract, or is the result of an over-all increase in the activity of noradrenergic nerves remains an unsettled question. Comparison between plasma levels in different veins may give valuable information.

It is also unknown whether the elevated plasma noradrenaline level is an etiological factor in DU disease or if it is merely a consequence of the disease. Further studies are needed to establish whether noradrenaline influences the stomach directly or indirectly by modifying the release of gastrointestinal hormones, changing local blood flow or altering the resistance of the mucosa to excess acid. However, such studies are difficult to perform since the elevation of noradrenaline in DU disease is small and therefore difficult to mimic by intravenous infusion; unphysiologically high concentrations are easily obtained. Also, it is possible that a prolonged period of increased plasma noradrenaline concentration may be the causative factor. A recent study by our own group suggests that the release of cholecystokinin, neurotensin, motilin and somatostatin is not altered in DU patients in response to MSF (88).

When comparing the insulin and MSF tests in DU patients 6 weeks after HSV no differences in the acid responses were obtained despite the fact that both adrenaline and noradrenaline levels were significantly higher during insulin hypoglycemia than during MSF. Acid secretion in response to insulin hypoglycemia has also been reported to be similar in adrenalectomized patients and normal volunteers despite very large differences in plasma adrenaline (84). The adrenalectomized patients also had basal noradrenaline levels three times the normal value and still BAO was the same. In studies by Christensen and Stadil (77) infusion of a low dose of adrenaline (5 ng/kg/min) resulted in an increase in gastric acid output whereas infusion of a high dose (50 ng/kg/min) did not change acid output.

In conclusion our studies show that there are no correlation between variations in plasma adrenaline or noradrenaline concentrations and acid secretion during basal or stimulated conditions .

3.2 Circulating catecholamines and serum gastrin

3.2.1 Background

Infusion of adrenaline or noradrenaline has generally been reported to increase serum gastrin levels in animals and man (33,41,42,77,89-91). In particular, adrenaline was shown to be a very potent stimulator to gastrin release in DU patients (42). Activation of the SAS in the cat by means of unloading of carotid baroreceptors results in an elevated plasma gastrin level which is abolished by adrenalectomy (92). Stimulation of the sympathetic nerves to the stomach in the same species reduces serum gastrin (26) and it also significantly inhibits the vagally induced gastrin release (93).

It has also been found that elevated plasma catecholamine concentrations, induced by exercise or respiratory acidosis, increase gastrin levels (94,95). However, this is contradictory to the finding by Hostein (96). Insulin-induced hypoglycemia causes a rise in plasma catecholamines and serum gastrin (41,84), the latter response being blocked by propranolol (83,97,98). Patients with phaeochromocytoma, who have greatly elevated plasma catecholamine levels, also have an increased serum gastrin concentration (89), which is normalized after surgery (99,100). Gastrin release due to gastric distension is blocked by propranolol (101) and the same applies to the increased serum gastrin levels after infusion of amino acids (102). On the other hand basal as well as food stimulated gastrin secretion was unaffected by propranolol (103) but the concentration of component III (gastrin-17) was reduced by β blockade (104). During our studies, mainly focused on circulating catecholamines and gastric acid secretion, serum gastrin levels have also been measured.

3.2.2 Results (I,II,V,VI)

Compared to healthy volunteers, serum gastrin concentration was elevated in DU patients both during basal conditions and in response to food (I). On the other hand no changes in serum gastrin occurred in DU patients during stimulation with MSF (II). Basal serum gastrin concentration was slightly depressed by β -blockade in asymptomatic DU patients whereas serum gastrin concentration during MSF was unaffected by β -blockade. The selective antimuscarinic compound pirenzepine did not change basal serum gastrin concentration significantly.

3.2.3 Comments

Although both plasma noradrenaline and serum gastrin levels seem to be increased in DU patients, our studies could not reveal any clear-cut correlation between plasma noradrenaline and serum gastrin. Neither was there a relationship between plasma adrenaline and serum gastrin, as suggested by Brandsborg et al (41). Pirenzepine, which depresses plasma noradrenaline, did not affect serum gastrin levels and B-blockade, which did not have any influence on plasma catecholamines, slightly depressed serum gastrin. There is, however, some evidence to show that insulin hypoglycemia causes an increase in serum gastrin levels (41,83,97,98) which might be associated with the concomitant elevation of plasma adrenaline (41). However, as shown by Järhult et al (84), "supraphysiological" increments in plasma adrenaline are necessary to cause this release of gastrin. The suggestion that insulin tests may be falsely positive due to extravagal adrenergic release of gastrin (105) still remains to be proven although the results of β -blockade (83,97,98) point in this direction.

3.3 Effect of surgical and pharmacological acid depression on circulating catecholamines in DU patients.

3.3.1 Background

It is generally agreed that DU patients, as a group, have increased levels of basal and stimulated acid secretion. The observation that DU patients have significantly increased levels of plasma noradrenaline (39, I) initiated studies to find out if surgical (HSV) or pharmacological (cimetidine and pirenzepine) reduction of acid secretion could depress plasma noradrenaline in these patients.

3.3.2 Results (II, IV - VI)

Seven patients with endoscopically verified DU disease were subjected to HSV. Each patient was investigated on 3 separate occasions; 6 weeks before operation and then 6 weeks and one year postoperatively. No ulcer recurrences were recorded during the first postoperative year. The MSF test was carried out on each occasion whereas the pentagastrin test was performed preoperatively and the insulin test 6 weeks after the operation. Fig 5 outlines the BAO and PAO values during the MSF test. BAO decreased from a preoperative value of 6.7 (2.0-8.6) mmol H⁺/h to a value of 1.9 (0-3.6) 6 weeks postoperatively ($p < 0.05$) and 2.0 (0.2-2.2) one year after HSV ($p < 0.05$). PAO was reduced from 25.9 (10.8-27.4) mmol H⁺/h to 2.8 (0.2-4.6) ($p < 0.01$) and 5.8 (3.1-9.1) ($p < 0.02$), respectively.

Basal plasma noradrenaline levels fell significantly ($p < 0.01$) from 2.14 (1.1-2.8) nM to 0.54 (0.49-0.98) 6 weeks after HSV, but it then almost returned to the preoperative level (1.54 (0.94-1.92)) after 1 year postoperatively. (Fig 6.) Plasma adrenaline and dopamine concentrations were unchanged during the period of study.

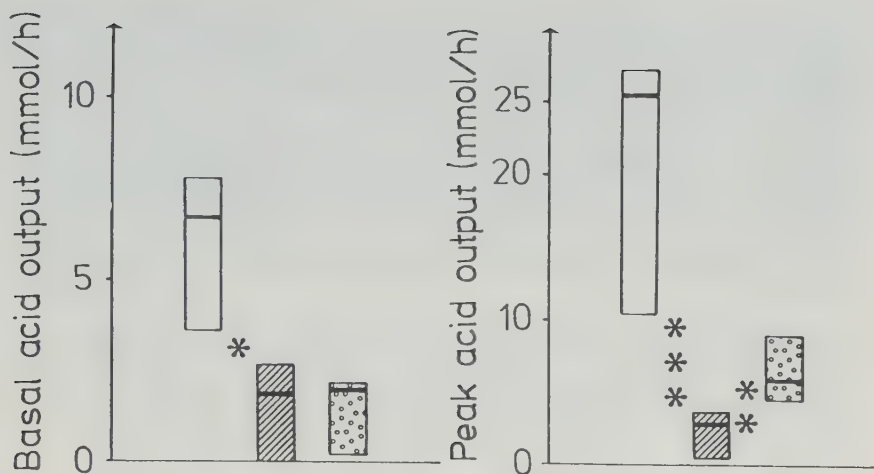


Fig 5. BAO and PAO in response to MSF before, 6 weeks (hatched bars) and 1 year (dotted bars) after HSV. * $p<0.05$, ** $p<0.02$ and *** $p<0.01$. Median and interquartile range shown.

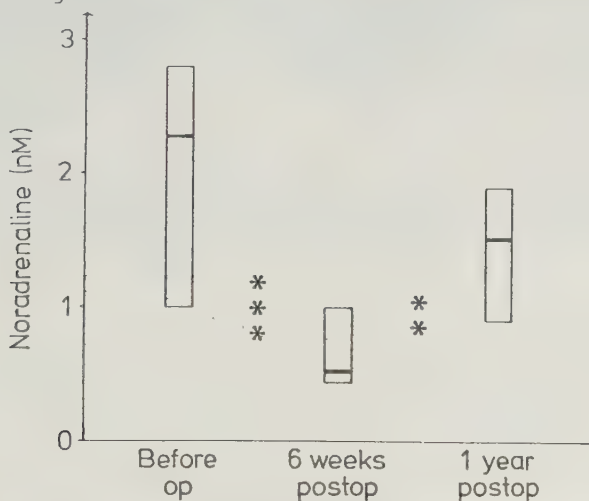


Fig 6. Plasma noradrenaline concentrations before, 6 weeks and 1 year after HSV. ** $p<0.02$ and *** $p<0.01$. Median and interquartile range shown.

Eight patients with chronic DU, but without symptoms, were given cimetidine (200 mg t.i.d. and 400 mg at night) for 10 days. Catecholamine levels measured before and on the last two days of medication did not differ despite the marked acid reduction induced by cimetidine.

Twenty DU patients with endoscopically proven active duodenal ulcers were treated with ranitidine (150 mg b.i.d.) for 4 weeks. Healing, verified by endoscopy, occurred in all cases. Blood samples for catecholamine analysis were obtained before treatment and after healing had occurred. Plasma noradrenaline and dopamine concentrations were significantly higher ($p < 0.02$) during active ulceration than after healing; on an average, the levels were 20 % higher.

In nine patients the injection of pirenzepine (10 mg i.v.) induced a significant but short-lasting fall in plasma noradrenaline concentration (Fig 7). Pirenzepine neither changed blood pressure nor plasma adrenaline, dopamine or serum gastrin and somatostatin concentrations. A slight increase in pulse rate was noted.

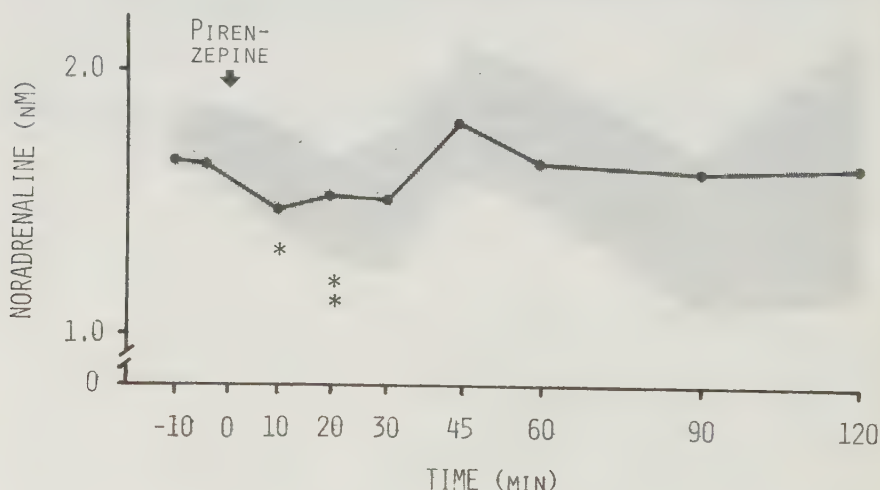


Fig. 7. Plasma noradrenaline concentrations before and after the injection of pirenzepine. Median and interquartile range shown. * $p < 0.05$ and ** $p < 0.02$.

3.3.3 Comments

In our study a significant decrease in plasma noradrenaline concentration after HSV was found. This observation should be compared to a previous study by Christensen (40) who found a decrease in plasma noradrenaline in only two out of six patients operated on with HSV (n=3) or selective gastric vagotomy with drainage (n=3). The discrepancy between our studies might be due to differences in the surgical procedures, in the time elapsing between operation and blood sampling and/or in the methods of catecholamine determination.

A drop in plasma noradrenaline levels after HSV might be explained in at least four different ways.

1. The increased noradrenaline levels in DU patients might depend on the fact that noradrenergic nerve fibers to the stomach are hyperactive in DU disease. Since the sympathetic fibers follow the vessels to the stomach, a drop in plasma noradrenaline will ensue when these vessels are ligated during HSV. The rise in plasma noradrenaline during the first postoperative year could then be explained by reinnervation of sympathetic nerves. However, the splanchnic area normally contributes relatively little to the overall plasma noradrenaline content, since the liver is an important tissue in the process of catecholamine degradation (106). Whether or not this is true also in DU disease remains to be shown.
2. Hypersecretion of acid per se may lead to a reflex increase in the activity of the SAS. Reduction of the acid secretion by HSV would then be expected to abolish this effect. Against this theory speaks the fact that no correlation was found between acidity and noradrenaline levels and that cimetidine, causing acid depression, had no effect on noradrenaline levels.
3. Afferent fibers from various receptors in the gut are part of the vagus (107) and if the activity of these fibers is increased in DU patients, leading to increased sympathetic activity, such an effect may be reduced by HSV. Again, reinnervation can be used as an explanation for the increase during the first postoperative year.
4. Nonspecific stress may be relieved after ulcer surgery. However, pain per se does not seem to be involved because elevated plasma noradrenaline concentrations were found in DU patients whose ulcers had healed and who experienced no pain. On the other hand patients had higher levels of noradrenaline during active ulceration than after healing had occurred. Probably this reflects an increase in sympathetic tone due to pain and discomfort during the active phase of the disease or reflects an increased activity of the sympathetic nerves per se, in turn contributing to the genesis of the ulcer.

In the studies described above it must be remembered that the drugs used also influence other physiological events, which may affect the plasma noradrenaline levels. For instance, cimetidine is known to reduce liver blood flow and to alter the metabolism of endogenous and exogenous substances (108). Pirenzepine, a selective antimuscarinic agent, is believed to have a direct effect on the SAS. The synaptic transmission between pre- and postganglionic sympathetic fibers is cholinergic and might therefore be influenced by anticholinergic drugs. The drug has a 50-fold higher affinity for muscarinic receptors in sympathetic ganglia than in other tissues (109). Also, the effect of pirenzepine on gastric acid secretion is reduced in animals pretreated with 6-hydroxydopamine (110) which is known to destroy sympathetic nerve endings (111). Postganglionic nerve endings possess receptors affecting transmitter release and synthesis. Many endogenous substances, including acetylcholine, are able to modify the noradrenaline release (112).

3.4 Effect of β -blockade on acid secretion.

3.4.1 Background

It has been repeatedly shown that infusion of the nonspecific β -agonist isoprenaline (31,73,79,113-115) decreases basal and stimulated acid secretion. This depression was blocked by the nonselective β -antagonist propranolol (31,114,115) and also by the selective β_1 antagonist practolol (31,115) but not by selective β_2 antagonists (31,115). β_2 agonists have also been shown to depress acid secretion (30,31,116). Studies of the effects of blockade of β -adrenoceptors on acid secretion and gastrin release have yielded conflicting results with one exception: Insulin-induced acid secretion is depressed by β -blockade (82,83,97,98). Pentagastrin-induced acid secretion was found to be depressed (117), unaffected (118-120) or increased (121) in response to β -blockade and basal acid output was found to be decreased (29,83), unchanged (97,98,117) or elevated (118).

Since insulin-induced acid secretion is depressed following β -blockade it would be of interest to study the effects of such a blockade also on MSF, a test that seems to be much more selective than the insulin test. Thus, the study described in paper V was performed.

3.4.2 Results (V)

Basal acid output was found to be significantly reduced by β -blockade (100 mg of propranolol in 6 DU patients) whereas peak acid output was unaffected.

3.4.3 Comments

This study confirms the notion that the SAS does not influence acid secretion during MSF in DU patients, at least not in vagally intact individuals. This fits well with the observation that MSF is not associated with alterations in plasma catecholamine concentrations (I, 87). As opposed to MSF (88) insulin-induced hypoglycemia also changes the blood levels of several gastrointestinal hormones (122,123). Therefore, and in consideration of the morbidity and mortality during insulin tests (124), MSF seems to be a better alternative for assessing vagal activity.

3.5 The effects of surgical sympathectomy in the rat.

3.5.1 Background

Sympathectomy - surgical, chemical (using 6-OHDA) or immunological - usually increases gastric acid output (22,125-130) although studies showing no changes after sympathectomy have been published (28,131,132). In earlier studies the completeness of the sympathectomy was usually not tested. With the development of the histochemical method of Falck and Hillarp (45) a semi-quantitative method for testing the completeness of denervation became available. The problem with chemical sympathectomy (6-OHDA) is that adrenergic nerve fibers everywhere in the body are destroyed, which might influence acid output indirectly.

The production of experimental ulcers is usually enhanced after sympathectomy (126,127,129,133) although the opposite effect has been reported also (52,134,135). Different methods for producing ulcerations and different species are probable explanations for this discrepancy. Christensen et al (136) have described an ulceroprotective effect of β -blockade using propranolol in the pylorus-ligated rat.

The effect of sympathectomy on the nerve supply to the digestive tract is known only to some extent. The proposed link between the noradrenergic nerve fibers and the enteric nervous system (137,138) makes such a study worthwhile. With the development of analyses for measurements of tissue catecholamines (46) the completeness of the surgical sympathectomy can be assessed by measuring the catecholamine levels.

3.5.2 Results (VII,VIII)

In normal rats the muscle layer of the acid producing part of the stomach carried the highest concentration of catecholamines. Surgical sympathectomy reduced the catecholamine levels to 10 or less than 10 per cent of the predenervation levels in the gastrointestinal tissues studied (Table III). Truncal vagotomy reduced gastric tissue catecholamines to about fifty per cent but did not change the catecholamine levels in extragastric tissues. Surprisingly, the

TABLE III

Noradrenaline concentration (ng/g) in various tissues after different denervation procedures.

Denervation	Fundus		Antrum		Duodenum whole wall	Midgut	Pancreas	Spleen
	mucosa	muscle	mucosa	muscle				
Control (n = 20)	274 (21) +++	1 143 (86) +++	471 (29) +++	668 (60) ++	696 (75) +++	615 (33) +++	927 (46) +++	1 283 (64) +++
Sympathectomy (n = 19)	6.6 (1.7) +++	40.2 (7.4) ++	15.4(3.3) +++	24.6 (5.6) +++	44.5 (2) n.s.	46 (3.6) n.s.	94 (8) n.s.	38.6 (6) n.s.
Vagotomy (n = 19)	114 (16) +++	334 (31) +++	207 (24) +++	331 (12) +++	863 (33) +++	639 (23) +++	726 (29) +++	1 260 (39) +++
Vagotomy and sympathectomy (n = 16)	26.1(4.8) +++	13.8 (1.9) +++	21.4(1.1) +++	28 (3.2) +++	3.1 (1.5) +++	1.4(0.2) +++	6.1(1.1) +++	5.1 (0.7) +++

Mean ($\pm$ SEM). + <0.05 ++ <0.02 +++ <0.01 n.s. = not significant compared to control rats

combined procedure (vagotomy and sympathectomy) resulted in significantly lower concentrations - compared to sympathectomy alone - in extragastric tissues only.

Fluorescence histochemistry, using the Falck & Hillarp method, revealed an absence of adrenergic nerve fibers in the denervated organs.

Pentagastrin- and histamine- induced acid secretion did not change after sympathectomy, while basal acid output increased significantly (Fig 8). Serum gastrin and basal gastric histidine decarboxylase were unaffected by sympathectomy.

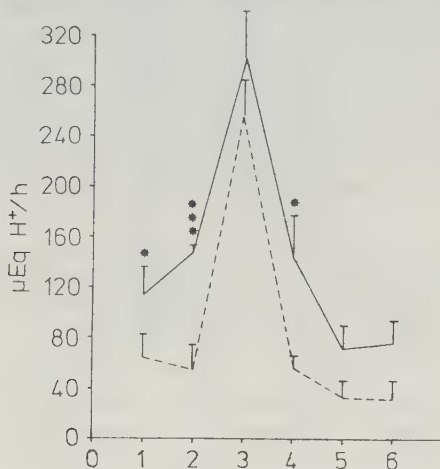


Fig 8. Acid output ($\mu\text{Eq}/\text{h}$) in response to pentagastrin($250 \mu\text{g}/\text{kg}$ b.w.) in sympathectomized (solid line) and control (broken line) rats. Mean $\pm$ S.E.M. * $p < 0.05$ and *** $p < 0.01$.

In paper VIII only a minor part is concerned with changes after sympathectomy. The peptidergic nerve fibers represent a quantitatively prominent part of the nerve supply to the stomach and seem to have a predominantly intrinsic origin; vagal denervation did not affect the density and distribution of the peptide - containing nerve fibers, and nerve cell bodies containing immunoreactive peptides were detected in the intramural ganglia. In the normal rat and mouse, a widespread distribution of somatostatin, gastrin/cholecystokinin (CCK), vasoactive intestinal peptide (VIP), substance P (SP), gastrin-releasing peptide (GRP), enkephalin and neuropeptide Y (NPY) nerve fibers could be demonstrated in the mucosa, submucosa and muscle coat of the gastric wall. However, sympathetic denervation resulted in the disappearance of mucosal and vascular NPY fibers leaving nerve fibers in smooth muscle as well as the other types of peptide containing nerve fibers unaffected.

3.5.3 Comments

As described in 3.4.1 and 3.5.1 studies on the role of the SAS using pharmacological stimulation or blockade and chemical sympathectomy have yielded conflicting results, the reason possibly being that these models influence sympathetic neurotransmission in general and to a varying degree. Upper abdominal sympathectomy appears to be a more selective way of studying the role of the sympathetic system in the control of upper gastrointestinal functions. Measurements of tissue catecholamines in this study show that the highest levels occur in the muscle layer of the acid producing part of the stomach. This is in accordance with the histological picture showing that the majority of the noradrenergic nerve terminals ramify around the neurones of the myenteric and submucous ganglia (16). Although fluorescence histochemistry revealed a complete absence of noradrenergic nerve fibers, surgical sympathectomy did not completely eliminate noradrenaline from the tissues. Conceivably this may be due to noradrenaline left in the vascular bed and/or the presence of noradrenergic intramural neurones. Studies of the catecholamine content in tissues after 6-hydroxydopamine treatment are in progress to see if a further reduction is possible.

Vagotomy reduced catecholamine levels in gastric tissues only, suggesting that the vagal adrenergic fibers (14,15) supply the stomach wall exclusively. The reason for the finding that the combined procedure (sympathectomy and vagotomy) led to reduced catecholamine concentrations in extragastric, but not in gastric tissues, remain unexplained.

The immunocytochemical studies after denervation indicate that the peptidergic nerve fibers are intramural in origin with the exception of mucosal and perivascular NPY fibers. Earlier studies have suggested that noradrenaline and NPY coexist in sympathetic nerve fibers (137,138). It seems as if NPY fibers are of two different types. This is in accordance with studies by Lundberg and Tatemoto (139) showing that an NPY-like peptide is present together with noradrenaline in vascular nerves of the cat submandibular gland but that noradrenergic nerves around exocrine acini are without NPY. The coexistence of noradrenaline and other neuropeptides has earlier been proposed in prevertebral ganglia (somatostatin-like peptide) (140) and enkefalin-like peptides have been found in the adrenal medullary gland (141). Little is known of the physiological properties of NPY. The peptide seems to have general vasoconstrictor actions (142).

Experiments performed by Ekelund (80) indicate a slight depressant effect of NPY on basal acid output in the rat but the addition of NPY to noradrenaline infusion does not change the depressant action of the latter compound (Fig 9).

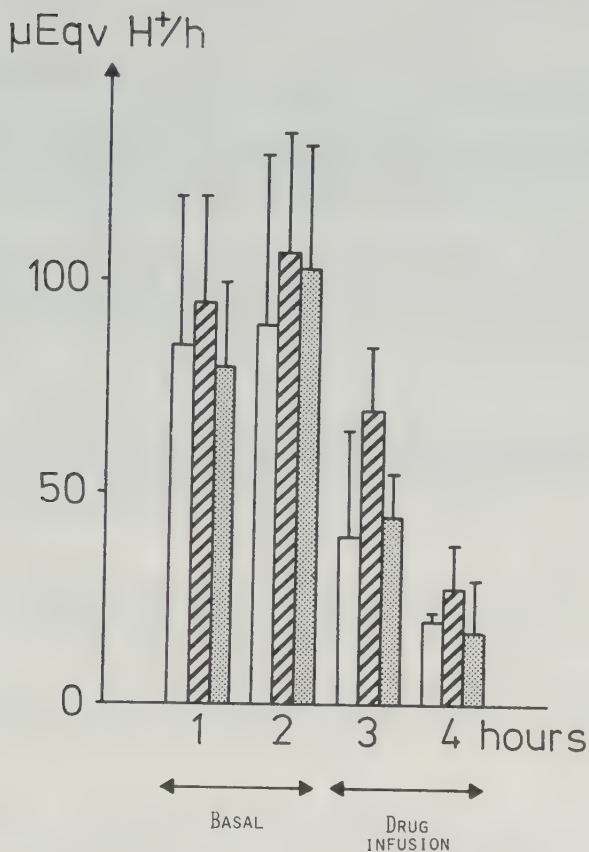


Fig 9. The effect of noradrenaline (open bars-50 $\mu\text{g/kg/h}$), NPY (hatched bars-10 $\mu\text{g/kg/h}$) or a combined infusion of the two hormones (dotted bars) on basal acid output ($\mu\text{Eqv H}^+/\text{h}$) in the rat.

3.6 Clinical considerations and possible pathways for future work

The extensive use of pharmacological compounds influencing the SAS, i.e. β -blockers, has not been reported to have any potential benefits or drawbacks in ulcer diathesis. This fact implies that the SAS does not have a major influence on ulcer formation.

When performing an HSV, a selective sympathectomy of the parietal cell mass is also conducted. It is possible that in some patients, if sympathectomy increases acid secretion, the vagotomy and the sympathectomy counteracts in such a way that the acid secretion is less decreased. This might explain a case of "incomplete vagotomy" where the surgeon is certain that the dissection was complete.

MSF did not evoke any increase in plasma catecholamines and no differences in acid secretion was found when a β -blockade was added. Therefore, and particularly when considering the morbidity and mortality reported during insulin tests (124), MSF seems to be a better alternative than insulin hypoglycemia for assessing vagal activity.

Although studies have earlier been performed using infusion of noradrenaline (Table I) measurement of the resulting plasma concentrations have not been performed. Therefore it is not known if the increments in plasma noradrenaline are within "the physiological range." Studies of gastric acid secretion and gastrin release during infusion of small doses of noradrenaline and adrenaline during basal and stimulated conditions in DU patients and healthy volunteers and simultaneous determination of plasma catecholamines are in progress and will possibly further clarify the role of these hormones in DU disease.

A more selective way of testing the influence of the SAS on gastric function has recently been described by Debas et al (143) using total devascularisation of the stomach with immediate revascularisation. In this way possible effects of denervated organs, other than the stomach, may be excluded.

Analyses of tissue catecholamine content from biopsy specimens of the gastric mucosa in DU patients before and after vagotomy and in healthy volunteers might be of value in assessing the role of the SAS in DU disease. Also sampling of blood from the portal system and comparison with peripheral venous catecholamine concentrations might show differences in DU patients compared to controls.

4. CONCLUSIONS

- * Patients with chronic duodenal ulcer (DU) disease have increased plasma concentrations of noradrenaline during basal conditions, during stimulation with food and during insulin hypoglycemia.
- * The elevated plasma noradrenaline concentration drops immediately after highly selective vagotomy (HSV) and gradually rises with time during the first year after operation.
- * Acid stimulation by insulin hypoglycemia - but not by modified sham feeding (MSF) - activates the sympatho-adrenal system with possible effects on acid output and gastrin release. Acid secretion during MSF is not affected by β -blockade. Thus it seems that MSF, but not insulin hypoglycemia, is a valid and selective procedure for assessing completeness of vagotomy.
- * The increased noradrenaline level in DU patients is not secondary to the acid hypersecretion since the levels of circulating catecholamines were not altered after acid depression by cimetidine.
- * Pirenzepine depresses plasma noradrenaline, possibly due to blockade of neurotransmission in sympathetic ganglia.
- * Extirpation of the coeliac and mesenteric ganglia in the rat resulted in greatly reduced catecholamine concentrations in the denervated stomach. Basal acid output was increased after such a selective sympathectomy, whereas the pentagastrin- and histamine-stimulated acid output was unaffected. No changes occurred in basal serum gastrin concentration or gastric mucosal histidine decarboxylase activity.
- * Noradrenergic fibers disappeared from the stomach after selective upper abdominal sympathectomy. With the exception of NPY fibers, peptide containing nerve fibers in the stomach were unaffected by surgical sympathectomy. Vascular and mucosal NPY fibers, but not NPY fibers to the smooth muscle, disappeared following denervation. The possible role of NPY in gastric physiology remains unidentified.

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Effects of Food on Plasma Catecholamine and Gastrin Levels in Patients with Duodenal Ulcer and Normal Volunteers

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Abstract. Changes in plasma concentrations of adrenaline, noradrenaline, dopamine and gastrin in response to a standard meal were studied in 6 normal volunteers and 8 patients with chronic duodenal ulcer (DU) disease. Before the meal plasma gastrin and norepinephrine, but not adrenaline or dopamine, were higher in DU patients than in the controls. Food induced significant increments in plasma gastrin and noradrenaline concentration in both groups, whereas plasma adrenaline and dopamine levels remained unchanged. Plasma gastrin and noradrenaline concentrations were higher in DU patients than in the normal controls both during and after the meal. The results do not support the hypothesis that adrenaline is involved in the pathogenesis of duodenal ulcer disease, whereas the role of the increased plasma noradrenaline concentrations in this disease remains unclear.

Infusion of adrenaline stimulates gastrin secretion in man [1, 5, 16] and the effect seems to be abolished by pretreatment with beta-blocking drugs [16]. However, the plasma concentration of adrenaline required to evoke gastrin release seems to be relatively high, at least in normal volunteers [12]. Brandsborg et al. [3] recently reported that duodenal ulcer (DU) patients have an increased level of plasma noradrenaline and that they respond to exogenous adrenaline with a higher gastrin output than healthy controls [4], implying that enhanced sensitivity to adrenaline is implicated in the patho-

genesis of DU disease. We have now investigated the possible relationship between catecholamines and gastrin in this disease by monitoring plasma adrenaline, noradrenaline, dopamine, and gastrin concentrations during a standard meal in normal volunteers and patients with chronic DU disease.

Material and Methods

6 healthy volunteers (median age 33 years, range 30–37 years: all males) and 8 patients with a history of peptic ulcer disease (median age 57 years, range 33–66 years: 1 female and 7 males) gave their advised con-

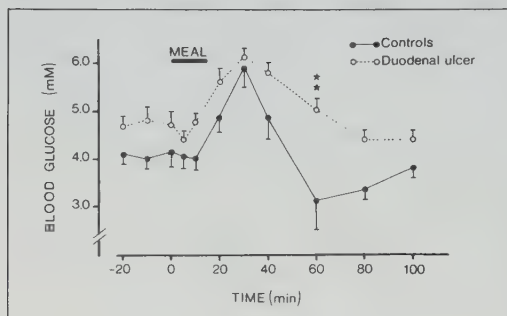


Fig. 1. Changes in blood glucose concentration in response to a standard meal in 8 patients with DU disease and 6 healthy controls. Asterisks indicate a significant difference between the two groups. ** $p < 0.01$.

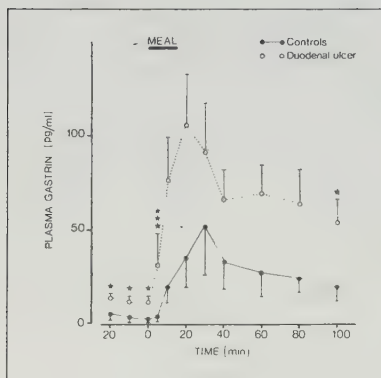


Fig. 2. Changes in plasma gastrin concentration in response to a standard meal in 8 patients with DU disease and 6 healthy controls. Asterisks indicate a significant difference between the two groups. * $p < 0.05$; *** $p < 0.001$.

sent to participate in the study. None of the volunteers had a history of peptic ulcer disease or of epigastric pain. All duodenal ulcers were established by both endoscopy and double contrast upper GI-examinations. At the time of the study the ulcers had healed and the patients experienced no symptoms suggestive

of DU disease. Except for antacids, the patients had received no medical treatment for 5 days before the investigation.

Each subject was investigated the morning after an overnight fast and prohibition of smoking for 12 h. A polyethylene catheter was implanted in an antecubital vein and used for collection of blood samples. The subjects were sitting comfortably in an armchair throughout the experiment. After a 30-min control period a meal comprising a sirloin steak, vegetables, and milk was served. The meal contained 39 g protein, 30 g fat, and 30 g carbohydrate with an energy content of 555 kcal (2.32 kJ). The meal was consumed during a 15-min period after which the subjects rested for another 85 min. Blood samples for determination of haemoglobin, haematocrit, glucose, gastrin, adrenaline, noradrenaline, and dopamine were withdrawn at intervals (fig. 1–4). Heart rate was recorded each time a blood sample was collected.

Glucose was measured using glucose oxidase. Plasma gastrin concentrations were determined by the radioimmunoassay technique described by Nilsson [14], using antiserum 2604-8 from Rehfeld's laboratory. The intra- and interassay coefficients are 11% and the antiserum reacts equally well with gastrin-17 and gastrin-34. Plasma concentrations of adrenaline, noradrenaline, and dopamine were measured using high-pressure liquid chromatography with electrochemical detection [10].

Statistical significance of difference was calculated using Wilcoxon's matched-pairs' signed-ranks test or, when appropriate, the Mann-Whitney U-test for unpaired data [15]. All values are expressed as mean $\pm$

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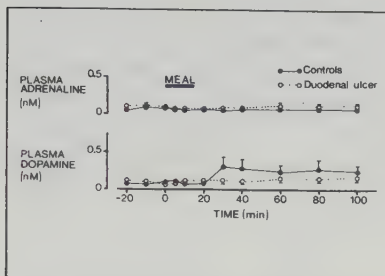
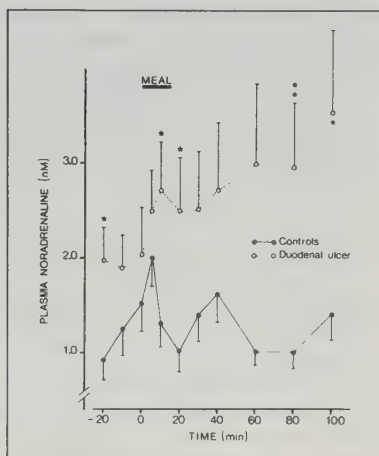


Fig. 3. Changes in plasma adrenaline and dopamine concentrations in response to a standard meal in 8 patients with DU disease and 6 healthy controls.

Fig. 4 Changes in plasma noradrenaline concentration in response to a standard meal in 8 patients with DU disease and 6 healthy controls. Asterisks indicate a significant difference between the two groups. * $p < 0.05$; ** $p < 0.01$.



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SEM. Basal catecholamine and gastrin concentrations (= in the control period before the meal) were calculated as the mean value of 3 different determinations in each individual.

Results

There were no differences in haemoglobin or haematocrit between the two groups, nor within each group, in response to food.

Heart rate at rest was 68 ± 1 beats/min in the control group and 64 ± 1 beats/min in DU patients. Heart rate rose significantly in both groups in response to food (74 ± 2 beats/min in normals and 71 ± 2 beats/min in patients with DU).

Mean blood glucose concentration before the meal was significantly higher in patients with DU (4.7 ± 0.2 mM) than in the controls (4.1 ± 0.1 mM; $p < 0.02$). Figure 1 shows that the extent of the rise in blood glucose in

response to the meal was closely similar in each group whereas the postprandial hypoglycaemia was more pronounced in the controls (blood glucose at 60 min after the meal being 3.1 ± 0.6 and 5.1 ± 0.3 mM, respectively; $p < 0.01$).

Mean plasma gastrin concentration before the meal was significantly higher in DU patients compared with the controls ($p < 0.02$). Figure 2 illustrates the changes in plasma gastrin in response to a meal in the two groups. The meal caused gastrin release in both groups, although the increment was more pronounced in the DU group. Plasma gastrin concentration remained elevated in the postprandial period in both groups, but was consistently higher in DU patients than in normals.

Changes in the mean plasma concentrations of adrenaline and dopamine are illustrated in figure 3. Plasma adrenaline was barely detectable in either group ($0.07 \pm$

0.01 nM in controls and 0.09 ± 0.02 nM in DU patients) and no increase was detected in response to the meal. There was no significant difference in mean plasma dopamine concentration during the control period before eating (0.09 ± 0.01 nM in the normal group and 0.10 ± 0.01 nM in the DU patients). No significant differences in plasma dopamine were noticed between the two groups in response to the meal, although a clear rise in dopamine concentration occurred in two normal volunteers, each of whom also displayed a pronounced postalimentary hypoglycaemia (3.0 and 2.8 mM, respectively).

The changes in mean noradrenaline concentrations before, during, and after the meal are shown in figure 4. Both at rest and before the meal, the plasma noradrenaline concentration was higher in DU patients (1.96 ± 0.22 nM) than in the normal group (1.25 ± 0.16 nM). Each group responded to the meal with a significant rise in plasma noradrenaline (peak increment 0.44 ± 0.14 nM in normals; $p < 0.05$, and 0.64 ± 0.12 nM in DU patients; $p < 0.02$), but there was no significant difference in the extent of this response between the two groups. After the meal the concentration of noradrenaline in the plasma fell rapidly towards the control level in normal subjects. In contrast, the plasma noradrenaline concentration remained elevated in DU patients after eating during the whole period of observation (= 1½ h after the meal).

Discussion

The sympathetic nervous system has attracted much less interest with regard to the pathogenesis of DU disease than its parasymp-

athetic counterpart. However, during recent years Brandsborg et al. [2, 4] have reported that DU patients have higher plasma concentrations of noradrenaline than normal subjects, and further, that the release of gastrin in these patients is much more sensitive to exogenous adrenaline and isoproterenol than in healthy controls. Our present study, using a highly sensitive method for the determination of plasma catecholamines, confirms that the plasma level of noradrenaline at rest is increased in patients with DU. In addition the study shows that DU patients, in comparison with healthy subjects, have higher plasma noradrenaline concentrations both during and after a meal. This increase in plasma noradrenaline concentrations might explain the significantly higher blood glucose levels observed in DU patients (see Results). In normal subjects most of the noradrenaline in the circulation seems to emanate from nerve terminals of postsynaptic adrenergic neurons. We suggest that the elevated plasma noradrenaline concentration in DU patients is due to an increased activity of these neurons rather than enhanced release of noradrenaline from the adrenal glands, since the plasma adrenaline concentration was unchanged. Whether there is a generalized increase in sympathetic tone or a selective hyperactivity in adrenergic nerves confined exclusively to the stomach (or gut) in DU patients remains to be shown. It is also possible that increased sympathetic tone compensates for vagal hyperactivity, which has been postulated to occur in DU patients [7], or that stimulation of gastrointestinal receptors, mediating their impulses via vagal afferents, evokes a reflex increment in sympathetic discharge.

Previous studies have demonstrated that the plasma gastrin concentration at rest is

increased in DU patients [3, 6, 9] and that their gastrin response to food is exaggerated [3, 13]. Our results confirm these findings. There is, however, no clear explanation of the difference between normal volunteers and DU patients. Some authors claim that the antrum contains an excess of gastrin cells in many DU patients [8] but this supposition is not generally accepted. It is highly unlikely that the elevated plasma noradrenaline concentration could contribute to the hypergastrinaemia in DU disease as neither endogenous [11] nor exogenous [5] increments in plasma noradrenaline concentration evoke any appreciable change in gastrin release. The hypothesis that the gastrin cells in DU patients have increased sensitivity to adrenaline [4] could explain the higher interdigestive plasma levels of gastrin in these patients than in healthy controls, but such mechanism can hardly be responsible for the meal-induced hypergastrinaemia. In the present study there was no change at all in plasma adrenaline concentration in association with ingestion and digestion of food, suggesting that the meal-induced rise in plasma gastrin in DU patients was not caused by this catecholamine *per se*. In our experience, the plasma concentration of adrenaline necessary to evoke a significant increase in plasma gastrin in man is relatively high and exceeds the normal adrenaline levels by 30–50 times [12]. However, such elevations of the plasma adrenaline concentration are seen only during severe stress situations. Whether or not adrenaline potentiates some unknown gastrin secretagogue in DU patients remains to be shown.

It is thus uncertain how adrenergic mechanisms could stimulate to hypersecretion of acid and gastrin, thereby contributing to the development of duodenal ulcers. It cannot

entirely be excluded that increased activity in the sympathoadrenal system is a separate phenomenon in DU patients without any causal relationship to the disease. If there exists a pathogenic role of this system we can only suggest that vascular effects of noradrenaline may be involved in the development of DU disease.

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Elevated Plasma Levels of Noradrenaline in Duodenal Ulcer

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The changes in plasma catecholamine and gastrin concentrations and gastric acid secretion in response to modified sham feeding were investigated in 7 duodenal ulcer (DU) patients before and 6 weeks after highly selective vagotomy (HSV). Both basal and peak acid secretion in response to modified sham feeding were significantly reduced by HSV. Basal plasma noradrenaline concentrations were elevated in DU patients but fell to normal values after HSV. Plasma adrenaline concentrations were within the normal range and did not change after HSV. Basal gastrin concentrations were significantly higher after HSV than before. Modified sham feeding caused no significant changes in plasma catecholamine and gastrin concentrations either pre- or postoperatively. It is concluded that noradrenaline is involved in, or reflects, DU disease.

The results of previous studies with adrenergic agonists and antagonists have suggested that beta-adrenoceptor stimulation enhances gastrin release in humans [1-4], whereas adrenergic stimuli have been reported both to potentiate [1-3, 5] and inhibit [6] acid secretion. Surprisingly enough, there are relatively few studies on the role of the sympatho-adrenal system in the pathogenesis of duodenal ulcer (DU) disease. An attractive hypothesis, postulated recently by Brandsborg and co-workers [7], is that the sensitivity of the gastrin cells to exogenous adrenaline is increased in DU patients. However, the plasma levels of this amine are barely detectable at rest [8, 9] and do not change during ingestion or digestion of food [9]. Plasma noradrenaline, on the other hand, seems to be markedly elevated in DU disease [10, 11], but infusions of this

catecholamine in healthy subjects lead only to a slight inhibition of gastric acid secretion [12]. In this study, a highly sensitive method for the determination of plasma catecholamines has been employed to investigate the relation between plasma adrenaline, noradrenaline, and gastrin concentrations, and gastric acid secretion before and after highly selective vagotomy (HSV) in a group of patients with DU disease.

Materials and Methods

Six males and 1 female (median age, 55 years; range, 33-65 years) with endoscopically proven DU disease and subject to HSV according to Amdrup [13] and Johnston [14] gave their informed consent to participate in the study. Each patient was investigated on 2 occasions: 6 weeks before and 6 weeks after HSV. At the time of these investigations, the patients experienced no epigastric pain and were receiving no medical treatment. Each investigation was performed the morning after an overnight fast and the subjects had abstained from smoking for the same period. They rested in an armchair throughout the experiment. A polyethylene cannula was implanted into an antecubital vein to permit collection of blood samples. A nasogastric tube was positioned in the stomach and gastric juice was then continuously aspirated by intermittent pump suction into containers which were changed at 15-min intervals. After a 60-min control period, a meal consisting of sirloin steak, potatoes, vegetables, and a glass of water was served. Each subject was instructed to chew the food and then spit it out; this process was completed within 10-15 min. Blood samples for determination of plasma adrenaline, dopamine, gastrin, glucose, and noradrenaline concentration were collected at intervals.

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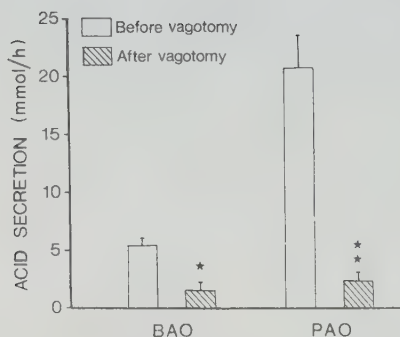


Fig. 1. Basal acid output (BAO) and peak acid output (PAO) in response to modified sham feeding in 7 patients with chronic duodenal ulcer disease before and 6 weeks after highly selective vagotomy. Asterisks indicate significant differences between pre- and postoperative values: * $p < 0.05$; ** $p < 0.01$.

The volume of gastric juice in each 15-min sample was recorded and a 5-ml aliquot was titrated to pH 7.0 using 0.1N NaOH in an automatic titrator (Radiometer, Copenhagen). Basal acid output (BAO) refers to the amount of acid secreted during the hour preceding stimulation. Peak acid output (PAO) was calculated as the acid secretion during the 2 highest consecutive 15-min periods after modified sham feeding multiplied by 2.

Plasma adrenaline, dopamine, and noradrenaline concentrations were measured using high pressure liquid chromatography and electrochemical detection [15]. Serum gastrin levels were determined by radioimmunoassay [16].

The statistical significance of differences between various variables was tested using the Wilcoxon matched pairs' signed-ranks test. Data have been expressed as mean values $\pm$ SEM.

Results

Figure 1 illustrates the effects of modified sham feeding on acid secretion before and 6 weeks after highly selective vagotomy in 7 patients with DU disease. Basal acid output decreased from a mean preoperative value of 5.4 ± 1.3 mmol/h to a postoperative value of 1.5 ± 0.6 mmol/h ($p < 0.05$). Peak acid output in response to modified sham feeding was reduced from 21.4 ± 2.7 mmol/h to 2.4 ± 0.7 mmol/h ($p < 0.01$).

Figure 2 shows the mean basal plasma concentrations of adrenaline, dopamine, and noradrenaline

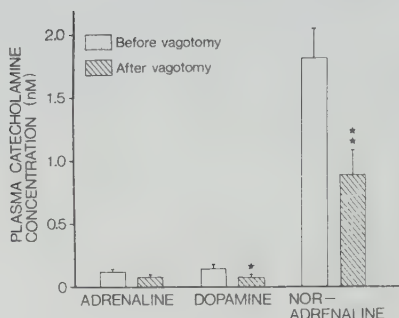


Fig. 2. Basal plasma adrenaline, noradrenaline and dopamine concentrations in 7 patients with chronic duodenal ulcer disease before and 6 weeks after highly selective vagotomy. Asterisks indicate significant differences between pre- and postoperative values: * $p < 0.05$; ** $p < 0.01$.

before and 6 weeks after HSV in the 7 DU patients. HSV caused a significant fall in the basal values of both dopamine ($p < 0.05$) and noradrenaline ($p < 0.01$). The mean basal plasma adrenaline concentration was quite low in these patients prior to the operation and was not significantly changed by HSV.

Modified sham feeding did not evoke any significant changes in plasma catecholamine concentrations either preoperatively or postoperatively. However, the mean plasma noradrenaline concentration was consistently higher before than after HSV throughout these sham feeding tests (Fig. 3).

Basal serum gastrin concentration rose from 223 ± 13 pg/ml to 255 ± 20 pg/ml after HSV ($p < 0.05$). There was no change in plasma gastrin concentration in response to modified sham feeding either before or after HSV.

Basal blood glucose concentration was unaffected either by HSV or sham feeding.

Discussion

The present finding that patients with duodenal ulcer disease have an elevated plasma noradrenaline concentration confirms the results of 2 previous reports [10, 11]. However, the source of this noradrenaline has not yet been established. As the mean plasma adrenaline concentration was completely normal in these patients, the data suggest—but do not prove—that the amine is released from some extra-adrenal tissue, possibly adrenergic nerve terminals. The normalization of plasma nor-

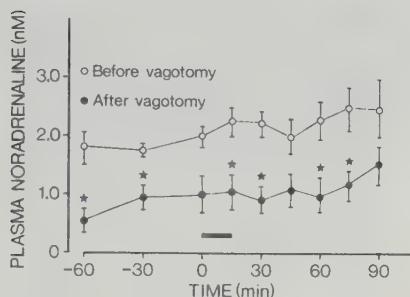


Fig. 3. Changes in plasma noradrenaline concentration in response to modified sham feeding in 7 patients with chronic duodenal ulcer disease before and 6 weeks after highly selective vagotomy. Asterisks indicate significant differences between pre- and postoperative values: * $p < 0.05$.

adrenaline levels by HSV may depend on one or more of the following possible explanations:

A. Adrenergic nerve fibers to the stomach may be hyperactive in DU disease. Sectioning of such adrenergic nerve fibers, some of which have been identified in the human vagi [17], during the operative procedure might then lead to a drop in plasma noradrenaline postoperatively. A recent investigation has revealed, however, that the parietal region stores only minute amounts of noradrenaline in humans [18].

B. Hypersecretion of acid per se may lead to a reflex increase in the activity of the sympatho-adrenal system and hence to a rise in plasma noradrenaline concentration. Reduction of the acid secretion by HSV would then be expected to abolish this effect by eliminating the irritant stimulus.

C. The vagi are known to contain a large number of afferent fibers emanating from a variety of receptors in the gut [19]. If the activity of such fibers is increased in DU patients and induces an overall increase in sympathetic activity, such an effect might be reduced if the afferent pathway were effectively interrupted by HSV. This possibility is supported by the likelihood that such fibers innervate receptors close to the site of gastric acid production.

D. It is also possible that the increased sympatho-adrenal activity which seems to characterize DU disease is due to nonspecific stress, which is relieved by surgery. However, pain per se does not seem to be involved because elevated plasma noradrenaline concentrations were found in DU patients whose ulcers had healed and who experienced no pain.

Our finding that the elevated plasma noradrenaline concentrations fell to normal after HSV contradicts observations obtained in a previous study [11] on 6 patients treated by HSV or selective gastric vagotomy with gastroduodenostomy. There is no obvious explanation for this anomaly.

It is not known whether noradrenaline contributes causatively to DU disease or is merely a consequence of the condition. Acutely, both exogenous and endogenous noradrenaline have been reported to inhibit the secretion of both gastrin and gastric acid [12, 20]. Furthermore, there is no difference between the gastric acid response to pentagastrin in sympathectomized and intact rats (Graffner, unpublished observations). Further studies are needed to discover whether noradrenaline influences the stomach indirectly by modifying the release of gastrointestinal hormones, changing the blood flow to the duodenum in some way, or altering the resistance of the mucosa to excess acid. It should also be borne in mind that nothing is known about the effects of elevated concentrations of noradrenaline in the plasma over prolonged periods, as might well occur prior to the development and diagnosis of a duodenal ulcer. There is no reason to suppose that the effects the catecholamine produces in the short term, under experimental conditions, provide a valid guide to the consequences that ensue when excessive amounts of noradrenaline are released into the circulation over periods of weeks or months.

It has recently been shown that ingestion of food significantly increases plasma noradrenaline concentrations both in DU patients and in healthy volunteers [9], corroborating findings that have been obtained in conscious laboratory animals [21]. As shown in the present study, modified sham feeding does not cause a perceptible rise in the concentration of noradrenaline in the plasma of DU patients, which suggests that the increase in sympathetic activity is not secondary to vagal stimulation of gastric acid secretion.

The mean basal plasma gastrin concentration was increased after HSV, confirming previous results by other groups [22–25]. The finding that plasma gastrin concentrations did not rise in response to modified sham feeding confirms recent results obtained by Olbe's group [26] but contradicts those reported by Mayer and co-workers [27].

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tory, Cambridge, has provided much valuable criticism of the manuscript.

Résumé

Les modifications de la concentration dans le plasma de la catécholamine et de la gastrine ainsi que les modifications de la sécrétion d'acide gastrique après repas d'épreuve simulé ont été étudiées chez 7 malades atteints d'ulcère duodénal avant la vagotomie hypersélective et 6 semaines après celle-ci.

La sécrétion basale et le pic de la sécrétion acide furent réduits après la vagotomie hypersélective. Les concentrations de la noradrénaline plasmatique qui étaient élevées chez les ulcéreux revinrent à la normale après l'intervention. En revanche, les taux de l'adrénaline plasmatique qui étaient voisins de la normale avant l'intervention ne varièrent pas après celle-ci. Les taux de la gastrine plasmatique furent sensiblement plus élevés après la vagotomie hypersélective. Cette étude permet d'affirmer que le repas d'épreuve simulée ne produit pas de modification significative des taux de la catécholamine et de la gastrine plasmatique avant ou après vagotomie hypersélective, mais, en revanche, l'intervention est suivie de la diminution du taux de la noradrénaline.

On peut conclure de l'ensemble de ces faits que la noradrénaline est impliquée dans la genèse de la maladie ulcéreuse ou qu'elle en constitue le reflet.

Abstracto

Los cambios en las concentraciones plasmáticas de catecolamina y de gastrina y en la secreción de ácido gástrico en respuesta a una comida fantasma (sham feeding), fueron investigados en 7 pacientes con úlcera duodenal (DU) antes y 6 semanas después de vagotomía altamente selectiva (HSV). Tanto la secreción basal como la secreción pico en respuesta a una comida fantasma modificada, fueron reducidas en forma significativa por la HSV. Las concentraciones plasmáticas de noradrenalina se encontraban elevadas en pacientes con UD, pero descendieron a valores normales después de HSV. Las concentraciones plasmáticas de adrenalina estuvieron dentro de límites normales y no cambiaron después de HSV. Las concentraciones basales de gastrina se hallaron significativamente más altas después de HSV que antes. La comida fantasma modificada no causó cambios significativos en las concentraciones plasmáticas de catecolamina y de gastrina, ni pre ni postoperatoriamente. Se concluye que la noradrenalina está involucrada en, o es un reflejo de la enfermedad ulcerosa duodenal.

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THE ROLE OF CATECHOLAMINES IN THE CONTROL OF GASTRIC ACID SECRETION DURING INSULIN HYPOGLYCAEMIA AND MODIFIED SHAM FEEDING

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Abstract. The modified sham feeding procedure has been advocated to replace the insulin test as the method of choice for the assessment of denervation after vagotomy. One of the reasons for this changing attitude is the assumption that insulin hypoglycaemia causes non-cholinergic stimulation of acid secretion, possibly mediated via the sympatho-adrenal system. This study has therefore compared the effects of these two different tests on acidity and release of catecholamines into the circulation in 7 duodenal ulcer patients 6 weeks after highly selective vagotomy. It was found that insulin, but not modified sham feeding, caused a marked increase in plasma adrenaline and noradrenaline concentrations whereas gastric acid secretion was of a similar magnitude in the two tests. The results suggest that the increased plasma catecholamine levels during insulin hypoglycaemia do not influence gastric acid secretion in duodenal ulcer patients.

Fifty years ago Simici, Popesco & Diculesco (1927) reported that hypoglycaemia, induced by the injection of insulin, was an effective stimulus to gastric acid secretion in man. This observation was later on adopted by Hollander, who developed it to a test to determine the completeness of vagal denervation (Hollander, 1946). Despite 35 years of work on the Hollander test it is still open to question whether or not gastric acid secretion in response to insulin solely reflects vagal activation. Insulin-induced hypoglycaemia is thus known to activate the sympatho-adrenal system with subsequent release of adrenaline and noradrenaline into the circulation (Brandsborg et al., 1975; Järhult et al., 1981*b*). Since adrenaline enhances gastrin secretion and, possibly, acid secretion (Stadil & Rehfeld, 1973; Christensen & Stadil, 1976*b*) it has been proposed that the sympathetic nervous system contributes to

the increased secretion of acid in response to insulin hypoglycaemia (Christensen & Stadil, 1976*a*). This notion is supported by the finding that beta-adrenoceptor blockade suppresses insulin-induced acidity in duodenal ulcer patients both before and after vagotomy (Kronborg et al., 1974). Other authors, however, report that combined alpha- and beta-adrenoceptor blockade do not appreciably modify gastric acid secretion in healthy volunteers (Hodge et al., 1972).

Modified sham feeding with the chew-and-spit technique has recently been shown to be a safe, easy and adequate way to induce vagal activation of gastric acid secretion in man (Mayer et al., 1974; Stenquist et al., 1978). The aim of this study was to discover whether this test alters plasma catecholamine levels in duodenal ulcer patients after highly selective vagotomy and to compare the results thereof with those obtained in response to insulin hypoglycaemia.

MATERIAL AND METHODS

Six males and one female (median age 55 years; range 33–56 years) with chronic duodenal ulcer disease proven by endoscopy gave their advised consent to participate in this study. All patients were operated on with highly selective vagotomy (HSV) as described in 1970 by Amstrup & Jensen and Johnston & Wilkinson. Each patient underwent a preoperative pentagastrin test and was also studied after the operation with the insulin and chew-and-spit tests.

All the tests were performed the morning after an overnight fast and tobacco abstinence. The subjects rested comfortably in an armchair throughout the experiment. A polyethylene cannula was inserted into an an-

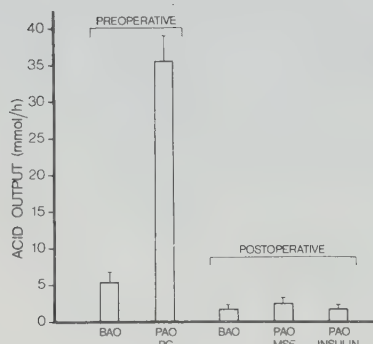


Fig. 1. Basal acid output (BAO) and peak acid output (PAO) in response to pentagastrin (PG), modified sham feeding (MSF) or insulin in 7 duodenal ulcer patients before and 6 weeks after highly selective vagotomy. There is a statistically significant difference between BAO pre- and postoperatively but not so between the postoperative PAO obtained with insulin or MSF.

tecubital vein to permit collection of blood samples. A nasogastric tube was positioned in the stomach and residual gastric juice aspirated. Gastric juice was then continuously aspirated by intermittent pump suction and the aspirates were collected at 15 min intervals over a period of three hours. After a 60 min control period insulin (Insulin Novo Actrapid®; 0.1 IU/kg b.wt. i.v.), pentagastrin (Peptavlon®; 6 µg/kg b.wt. s.c.) or a meal consisting of shredded sirloin steak, potatoes, vegetables and a glass of water was given. The patients were carefully instructed to chew the food and then spit it out. The meal was finished within 10–15 min. Blood samples were withdrawn every 15 min for the determination of blood glucose and plasma adrenaline, noradrenaline and dopamine concentrations.

The volume of gastric juice in each 15 min sample was recorded and a 5 ml aliquot titrated to pH 7.0 using 0.1 N NaOH in an automatic titrator (Radiometer, Copenhagen).

Blood glucose concentration was measured with glucose-oxidase. Plasma adrenaline, noradrenaline and dopamine concentrations were measured using high pressure liquid chromatography with electrochemical detection (Hallman et al., 1978).

The basal acid output (BAO) refers to the acid output recorded during the hour preceding stimulation. The peak acid output (PAO) was calculated as the two highest consecutive 15 min periods after stimulation multiplied by two.

Statistical significances of differences between various variables were tested with Wilcoxon's matched pairs signed-ranks test. Data are expressed as mean values $\pm$ SEM.

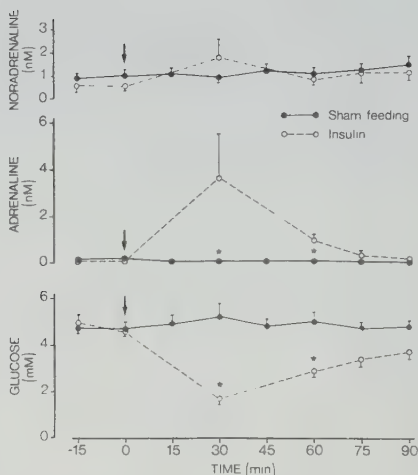


Fig. 2. Changes in plasma noradrenaline and adrenaline and in blood glucose concentrations in response to modified sham feeding and insulin in 7 patients with duodenal ulcer disease 6 weeks after highly selective vagotomy. Arrows indicate start of stimulation and asterisks indicate significant differences between the two tests ($p < 0.02$).

RESULTS

The changes in gastric acid secretion in response to insulin and modified sham feeding (chew-and-spit technique) in duodenal ulcer patients 6 weeks after the performance of highly selective vagotomy (HSV) are demonstrated in Fig. 1. HSV effectively reduced basal acid output (5.4 ± 1.3 mmol/h vs. 1.5 ± 0.6 mmol/h; $p < 0.05$). The preoperative peak acid output after pentagastrin was 35.2 ± 3.8 mmol/h and the postoperative PAO values were 2.4 ± 0.7 mmol/h (modified sham feeding) and 1.6 ± 0.8 mmol/h (insulin). There was no statistical difference between the post-vagotomy PAO value as assessed by insulin or modified sham feeding.

Insulin caused a significant ($p < 0.02$) fall in blood glucose concentration with a nadir value of 1.7 ± 0.2 mM after 30 min (Fig. 2). Concomitantly, there was a significant ($p < 0.02$) rise both in plasma adrenaline and noradrenaline concentrations. These alterations in blood glucose and plasma catecholamine concentrations were normalized 2 h after the start of stimulation. By contrast, no changes in blood glucose or plasma noradrenaline

and adrenaline concentrations occurred in response to the chew-and-spit test. Plasma dopamine concentration, not shown in the figures, remained unchanged in either test.

No patient experienced any side-effects during either test.

DISCUSSION

Hollander's insulin test is widely accepted as the method of choice for determination of the vagally induced gastric acid secretion. However, the results of the Hollander test are sometimes difficult to interpret and it has been claimed that these difficulties depend on the fact that the hypoglycaemia also evokes non-cholinergic stimulation of acid secretion. Injections of insulin are known to induce a profound activation of the sympatho-adrenal system, resulting in elevated plasma levels of adrenaline and noradrenaline (Brandsborg et al., 1975; Järhult et al., 1981*b*; see also Fig. 2) and to release a variety of different hormones (Hökfelt et al., 1978; Järhult et al., 1981*a*), some of which might lead to non-cholinergic inhibition or promotion of gastrin and acid secretion. However, the present results showed that the net effect of these substances on gastric acid secretion is negligible and that the PAO value does not differ from that obtained in response to 'pure' vagal stimulation by modified sham feeding. These findings thus contradict the theory that adrenaline stimulates gastric acid secretion either directly or indirectly via adrenaline-induced gastrin release (Stadil & Rehfeld, 1973). Instead they support our previous observation that adrenalectomized subjects secrete similar amounts of gastric acid as healthy volunteers in response to insulin (Järhult et al., 1981*b*). However, this observation was made in humans with vagally intact stomachs and it might be possible that adrenaline stimulates gastric secretion in the vagally denervated stomach.

This study has demonstrated that the chew-and-spit technique is a completely non-adrenergic stimulus to gastric acid secretion. On the other hand, it is probably not a purely cholinergic stimulus since atropine (30 µg/kg b.wt.) only inhibits the response by about 65% (Stenquist et al., 1979), i.e. an inhibition similar to that found during insulin hypoglycaemia using atropine at a dose of 15 µg/kg b.wt. (Farooq & Walsh, 1975).

The present investigation has confirmed previous observations that modified sham feeding

with the chew-and-spit technique is an easy procedure with minimal side effects (see Stenquist et al. 1978, 1979). These findings contrast with the fact that the insulin test occasionally may cause coma and death (Read et al., 1970). With regard to the effect on acid secretion response to modified sham feeding or insulin at a dose of 0.1 IU/kg b. wt. the present study confirms the results of a previous investigation (Stenquist et al., 1978) that these two tests evoke the same amount of acid. However, the number of patients is small and no definitive conclusions concerning the effect on acidity should therefore be made. Our results support the notion that the chew-and-spit technique is a more selective test of vagal activity than insulin. We therefore recommend that the chew-and spit technique replaces the insulin test in the postoperative assessment of the completeness of vagotomy.

ACKNOWLEDGEMENTS

This study was supported by grants from Maggie Stephen's Fund, the Swedish Medical Research Council (04X-2330) and the Torsten and Ragnar Söderberg's Foundation. The skilful technical assistance provided by Mrs Britt Englesson and Mrs Ulla Enberg is gratefully acknowledged.

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Is there a relationship between gastric acid secretion
and plasma catecholamines in duodenal ulcer disease ?

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Short title: Catecholamines and duodenal ulcer.

Key words. Adrenaline * Noradrenaline * Digestion *
Cimetidine * Sympatho-adrenal system *
Vagotomy * Duodenal ulcer

Abstract

Plasma adrenaline, noradrenaline and dopamine concentrations have been analyzed in three different groups of duodenal ulcer (DU) patients under various regimens of acid reduction in an attempt to find out if acidity per se has any influence on plasma catecholamine concentrations. Treatment with cimetidine, which reduced gastric acidity to the same level as after highly selective vagotomy (HSV), in patients with asymptomatic chronic DU disease increased plasma noradrenaline concentrations insignificantly. In a group of DU patients subjected to HSV, plasma noradrenaline levels were significantly higher one year than six weeks after operation despite the fact that basal acid output was the same. In a third group of DU patients plasma noradrenaline and dopamine levels were found to be 20% higher during the active, untreated ulceration than after four weeks of ranitidine treatment when the ulcer had healed. It is concluded that the elevated plasma noradrenaline levels found in DU patients are not caused by the hypersecretion of acid per se, nor seems there to be any general correlation between acidity and the catecholamine levels in peripheral plasma.

Patients with duodenal ulcer (DU) disease have elevated plasma levels of noradrenaline (1-4) and their gastrin cells seem to be more sensitive to stimulation by adrenaline than those in normal subjects (5). On the other hand, the effects of noradrenaline on plasma gastrin in humans seems to be dose-dependent (6). The effect of vagotomy on plasma catecholamine concentrations is not clearly established; one study - using a radioenzymatic method for catecholamine determination - has shown an unchanged noradrenaline concentration (7) whereas a recent investigation indicated that plasma noradrenaline was significantly decreased 6 weeks after highly selective vagotomy (HSV) (4).

The purpose of this study was to see if 1) A decrease in acidity, using H_2 -receptor blockade, alters plasma catecholamine levels in the same direction as HSV; 2) The observed decrease in plasma noradrenaline concentration 6 weeks after HSV is present also one year postoperatively; and 3) plasma catecholamine levels are higher during an active ulceration than after healing of the ulcer.

Materials and methods

The studies were approved by the Ethical committee of the University of Lund and all patients gave their advised consent to participate in the investigations.

All subjects were investigated in the sitting position the morning after an overnight fast and prohibition of smoking for 12 h. A polyethylene catheter was implanted in an antecubital vein. In some patients a nasogastric tube was positioned in the stomach and gastric juice was then aspirated by intermittent pump suction into containers that were changed every 15 min. At least 30 min elapsed between the introduction of the catheter and tube and the first blood sample. Plasma concentrations of adrenaline, noradrenaline and dopamine were measured using high pressure liquid chromatography with electrochemical detection (8).

All patients had a history of peptic ulcer disease of more than a year and the duodenal ulceration was verified by endoscopy in each case. The relation between catecholamines and gastric acid secretion was examined in three groups of patients as follows:

1. A group of DU patients (n=8, median age 52 years) without epigastric symptoms at the time of the study were treated with cimetidine (Tagamet R, SKF; 1 g daily) for ten days. Two blood samples for catecholamine determination were withdrawn on two consecutive days preceeding treatment and on the last two days of treatment, when 200 mg of cimetidine was administered 2 h prior to the sampling. This dose of cimetidine is known to depress basal and stimulated acid secretion to the same low levels as those obtained after HSV (9).

2. A group of DU patients (n=7, median age 55 years) who previously had undergone the modified sham feeding (MSF) test before and than 6 weeks after HSV (4) was investigated one year postoperatively. At that time all patients were without epigastric symptoms. This group of patients was again subjected to the MSF test. Blood samples for determination of plasma catecholamine levels were taken at intervals before, during and after the MSF test.

3. A third group of DU patients (n=20, median age 48 years) with active duodenal ulcerations verified by endoscopy was treated with ranitidine (Zantac R, Glaxo) at the dose of 150 mg twice daily for 4 weeks. After treatment, healing was assessed by endoscopy. Blood samples for catecholamine analysis were obtained before treatment and then again after healing had occurred. In this group all samples were stored at -20°C for 2 years before determination of plasma catecholamine concentrations. Due to possible degradation of the catecholamines with time, only the ratio between the values obtained before and after treatment is presented.

Data are expressed as median values and interquartile range. Wilcoxon's matched pairs signed-ranks test was used for calculation of statistical significance.

Results

Plasma adrenaline, noradrenaline and dopamine concentrations were the same before and during cimetidine treatment (fig 1).

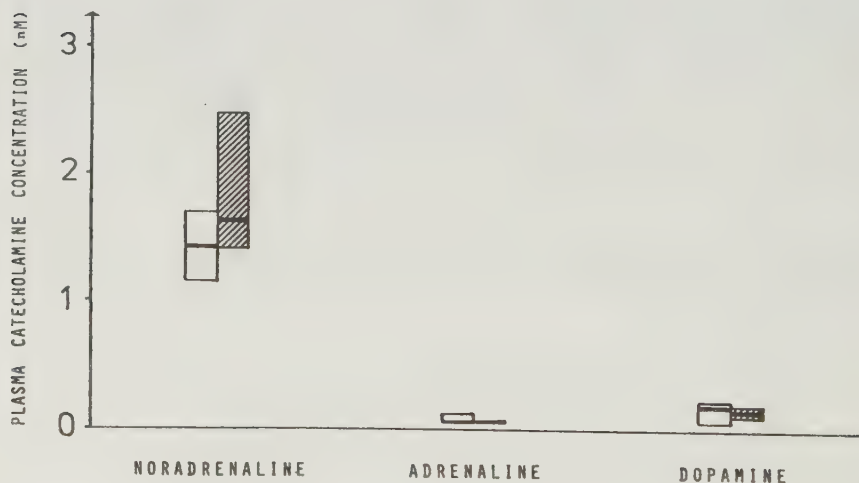


Fig 1 Plasma levels of adrenaline, noradrenaline and dopamine in 8 patients with duodenal ulcer disease before and during treatment with cimetidine at a daily dose of 1 g. Median values and interquartile range are shown. Open bars indicate control values and hatched bars indicate values during treatment.

The elevated plasma levels of noradrenaline in DU patients were significantly decreased 6 weeks after HSV. In comparison to this first postoperative value, a significant ($p < 0.02$) increase in plasma noradrenaline could be observed one year after operation although the noradrenaline values still were significantly ($p < 0.05$) lower than the preoperative ones (fig 2). No changes occurred in plasma adrenaline or dopamine concentrations in either of the postoperative controls. Basal acid output one year postoperatively was not different from the value observed 6 weeks after HSV. However, peak acid output after MSF was significantly ($p < 0.02$) higher one year after operation than 6 week postoperatively (fig 3). No correlations existed between the catecholamines and acid secretion.

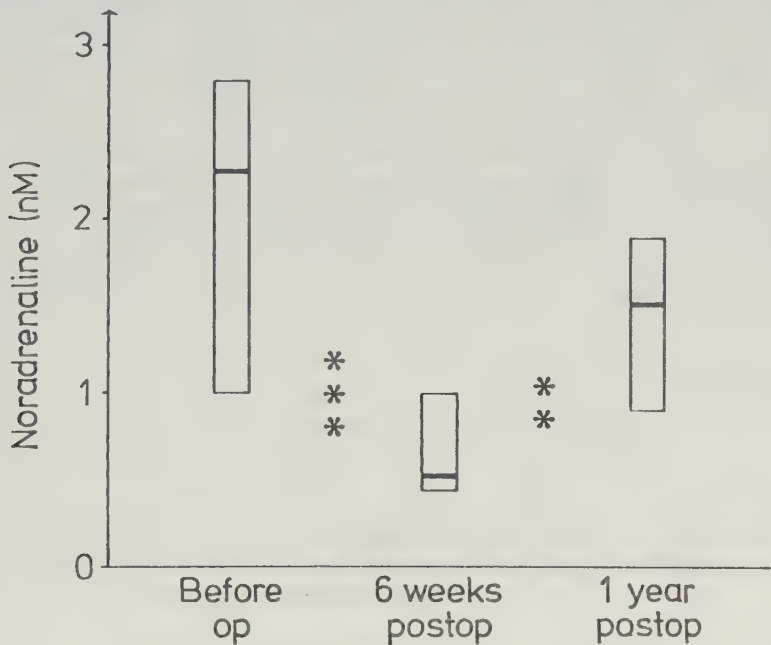


Fig 2 Plasma noradrenaline concentrations before, 6 weeks and 1 year after highly selective vagotomy in 7 duodenal ulcer patients.
** indicate $p < 0.02$ and *** indicate $p < 0.01$ Median and interquartile range shown.

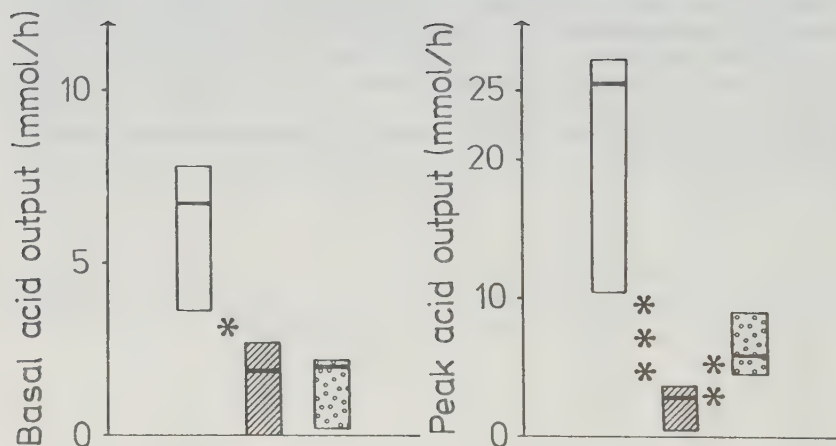


Fig 3 Basal and peak acid output in response to modified sham feeding before, 6 weeks (hatched bars) and 1 year (dotted bars) after highly selective vagotomy in 7 duodenal ulcer patients.* $p < 0.05$, ** $p < 0.02$ and *** $p < 0.01$. Median and interquartile range shown.

Plasma noradrenaline and dopamine concentrations were significantly ($p < 0.02$) higher during active ulceration than after healing; On an average, the plasma catecholamine levels were 20 % higher during the active phase of the disease.

Discussion

Controversies still exist with regard to the role of the sympathetic nervous system in duodenal ulcer disease. Our former studies have shown that the elevated plasma noradrenaline levels in patients with chronic DU disease were normalized 6 weeks after highly selective vagotomy (2) and this finding prompted us to investigate if a reduction of acidity per se may decrease plasma noradrenaline levels. However, we could not detect any significant alteration in either of the plasma catecholamines during peroral treatment with cimetidine which is known to cause an equally pronounced depression of acid secretion as HSV (9). It was recently reported in a preliminary form (10) that cimetidine increased plasma noradrenaline - but not adrenaline - levels in healthy volunteers (0.89 nM to 1.10 nM) and the authors speculated that cimetidine might depress the hepatic elimination of noradrenaline. However, it seems difficult to explain the rise in plasma noradrenaline merely by a disturbance of its elimination since plasma adrenaline, which uses the same metabolic pathways, seems unchanged by cimetidine (10, present study).

The increase in basal plasma noradrenaline levels by time after surgery (fig 2) is difficult to explain. Thus, basal acid secretion was the same 6 weeks and 1 year after HSV and the patients were without symptoms suggestive of ulcer recurrence on either occasion. It is tempting to speculate, however, that the initial reduction of plasma noradrenaline levels after HSV is caused by sectioning of sympathetic nerve fibers to the parietal region of the stomach which probably occurs when HSV is performed (Hottenrott, personal communication). Using this theory, the rise in plasma noradrenaline between 6 weeks and 1 year postoperatively could be explained by reinnervation of these nerve fibers. Against this speculation stands the fact that the splanchnic area contributes relatively little to the overall plasma catecholamine content, since the liver is an important, although not essential, tissue in the process of catecholamine degradation (11). It is important to notice that no correlation existed between gastric acid secretion and either catecholamine studied, neither before nor after vagotomy (2,4,12, present study). Taking into consideration that only minimal changes in noradrenaline levels occur in the presence of a strong antisecretory drug - cimetidine - it is reasonable to assume that the elevated noradrenaline levels in duodenal ulcer patients are not caused by the hypersecretion of acid per se.

The higher plasma levels of noradrenaline during an active ulceration than after healing might be explained by the fact that the patients experienced pain and epigastric discomfort in the former situation but it cannot be entirely excluded that the higher plasma noradrenaline concentration at the time for the active ulceration reflects an increased activity in the sympathetic nerves, in turn contributing to the genesis of the ulcer. However, it has previously been shown that infusion of noradrenaline in man, if anything, has a slightly inhibitory effect on gastric acid secretion (6).

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The effect of β -blockade on gastric acid secretion, gastrin release and plasma catecholamine concentrations during modified sham feeding in duodenal ulcer patients.

Short title: β -blockade and modified sham feeding.

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Abstract

The effects of β -blockade (propranolol 100 mg orally) on gastric acid output and on circulating levels of gastrin, adrenaline, noradrenaline and dopamine during modified sham feeding (MSF) were investigated with a randomized, double-blind method in 6 patients with asymptomatic duodenal ulcer (DU) disease. No differences occurred in peak acid output during MSF while basal acid output was significantly suppressed by β -blockade whereas peak acid output was unaffected. Basal gastrin concentration was lower during β -blockade but rose in response to MSF. Without β -blockade serum gastrin levels were unaffected by MSF. Plasma catecholamine concentrations were not affected by the β -blockade. It is concluded that acid output and gastrin release in response to modified sham feeding, unlike to insulin hypoglycaemia, is not influenced by β -adrenoceptor blockade.

Key words: β -blockade; gastric acid secretion; gastrin; insulin test; modified sham feeding; sympatho-adrenal system.

Acknowledgements

The authors are grateful to Mrs B. Sunden for her skilful technical assistance.

This study was supported by grants from the Medical Faculty, University of Lund and Maggie Stephens Fund.

The insulin test has for many years been the method of choice for evaluating the completeness of vagotomy (1). However, it has been shown repeatedly that insulin hypoglycemia also activates the sympatho-adrenal system (2,3), the effect of which may be to modulate gastric acid secretion and gastrin release (2,4). Further support for the idea that adrenergic mechanisms are important comes from studies showing that β -blockade decreases acid secretion and gastrin release in response to insulin-induced hypoglycemia (5-9).

Since insulin hypoglycaemia is an unspecific stimulus to gastric acid secretion and occasionally causes complications (10), it has been suggested that modified sham feeding (MSF), by means of the chew and spit technique, should replace the insulin test in the assessment of the completeness of vagotomy (11,12). The aim of the present study was to assess the effect of β -adrenoceptor blockade on gastric acid output, gastrin release and plasma catecholamine concentrations during modified sham feeding.

Materials and methods

Six patients (4 males and 2 females, median age 48 years, range 33-63) with a history of chronic duodenal ulcer (DU) disease for more than 1 year gave their advised consent to participate in this study. All patients had endoscopic evidence of duodenal ulcer but at the time of the investigation they were without symptoms suggestive of active ulceration. The study was approved by the Ethical committee of the University of Lund.

All tests were performed the morning after an overnight fast and tobacco abstinence. The subjects rested comfortably in an armchair throughout the experiment. A polyethylene cannula was inserted into an antecubital vein to permit collection of blood samples. A nasogastric tube was positioned in the stomach and residual gastric juice aspirated. By intermittent pump suction gastric aspirates were collected at 15 min intervals over a period of 135 min. After a 60 min control period a meal consisting of shredded sirloin steak, potatoes, vegetables and a glass of water was given. The patients were carefully instructed to chew the food and then spit it out. The meal was finished within 15 min. Blood samples for determination of serum gastrin and plasma adrenaline, noradrenaline and dopamine concentrations were withdrawn twice during basal conditions. Immediately after the meal another blood sample for determination of serum gastrin concentration was taken.

All patients received orally, in a random and double blind fashion with an interval of at least 3 days, either 100 mg of propranolol or placebo tablets 2 h before the test. Since propranolol has its peak plasma concentration 2 h after oral intake and a half-life in plasma of 3 h (13) this type of administration will lead to a coincidence between the peak plasma concentration of propranolol and the performance of the modified sham feeding test.

The volume of gastric juice in each 15 min sample was recorded and a 5 ml aliquot titrated to pH 7.0 using 0.1 N NaOH in an automatic titrator (Radio-meter, Copenhagen). Basal acid output (BAO) refers to the acid output recorded during the hour preceding stimulation. Peak acid output (PAO) was calculated as the two highest consecutive 15 min periods after stimulation multiplied by two.

Plasma adrenaline, noradrenaline and dopamine concentrations were measured using high pressure liquid chromatography with electrochemical detection (14). Serum gastrin concentrations were measured with radioimmunoassay (15).

Statistical significances of differences between various variables were tested with Wilcoxon's matched pairs signed-ranks test. Data are expressed as median and interquartile range.

Results

The changes in gastric acid secretion in response to modified sham feeding are shown in fig 1. BAO was significantly reduced by β -blockade ($p < 0.05$) whereas the PAO values were unaffected.

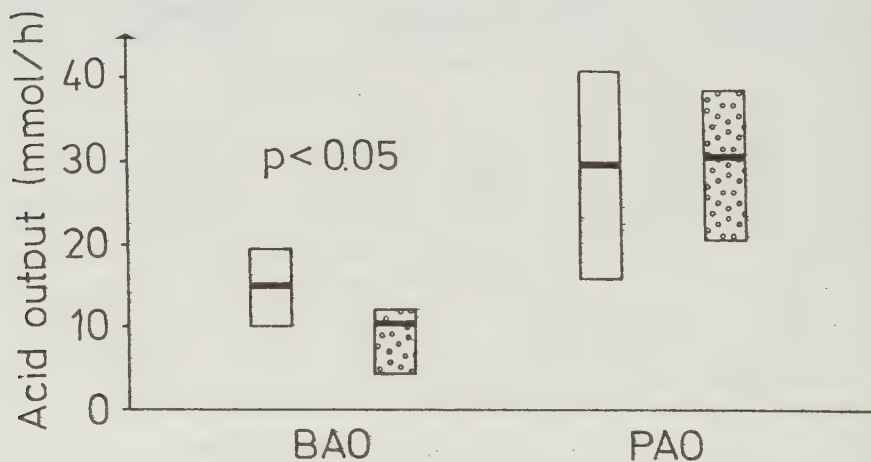


Fig 1 Basal acid output (BAO) and peak acid output (PAO) in response to modified sham feeding in 6 patients with DU disease. Dotted bars indicate acid output during β -blockade and open bars indicate acid output without β -blockade. Median values and interquartile ranges are shown.

Basal serum gastrin levels were reduced ($p < 0.01$) by β -blockade whereas the gastrin levels did not differ from that of controls immediately after modified sham feeding (Fig 2). In propranolol-treated, but not in untreated patients, the serum gastrin concentrations rose significantly ($p < 0.05$) in response to MSF.

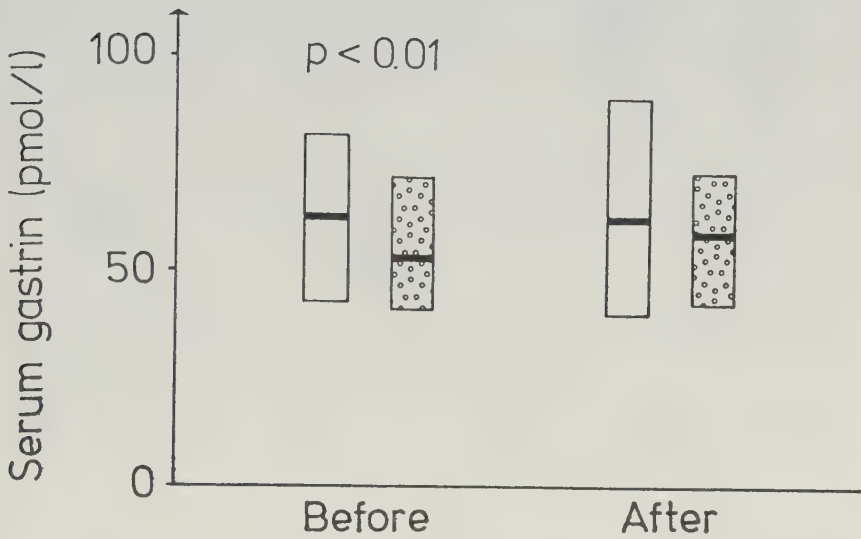


Fig 2 Serum gastrin levels before and after modified sham feeding in 6 patients with DU disease. Dotted bars indicate gastrin levels during β -blockade and open bars indicate serum gastrin concentrations in the untreated state. In propranolol-treated patients serum gastrin concentrations rose significantly ($p < 0.05$) in response to MSF. Median values and interquartile ranges are shown.

Plasma levels of adrenaline, noradrenaline and dopamine were unchanged by β -blockade (fig 3). As previously reported, basal plasma noradrenaline concentrations were higher in the DU patients than in normal healthy volunteers (16, 17, 18).



Fig 3 Plasma concentrations of adrenaline, noradrenaline and dopamine in the fasting state in the presence (hatched bars) or absence (open bars) of β -blockade in 6 patients with DU disease. Median values and interquartile ranges are shown.

Discussion

β -adrenoceptor agonists and antagonists have been widely used in studies of the role of catecholamines in gastric physiology. From these investigations it appears that β -agonists stimulate acid secretion and gastrin release (2, 4, 5). However, the results from studies with β -antagonists are conflicting both in quantitative and qualitative terms, possibly due to differences in administration dosage and pharmacological profiles of the different compounds used. It must also be born in mind that the extra-gastric effects of the β -blockers might cause secondary changes in gastric acid output and gastrin release.

Using the unselective β -antagonist propranolol, basal acid output seems to be decreased or unchanged both in normal subjects and in patients with DU disease (5-7, 19-20, present study), although a slight increase in BAO has also been reported (21). Stimulation of acid secretion with pentagastrin is unaffected (21,22) or depressed (20) by propranolol in man. In all reports insulin-induced acid secretion is depressed during unselective β -blockade (5-8). However a combined alpha and β -blockade has no effect on acid secretion (23).

Thus activation of the gastric β -adrenoceptors stimulates acid secretion and blockade of the β -adrenoceptors depresses both basal and stimulated acid secretion, particularly during insulin-induced hypoglycemia. MSF is an exception in that acid secretion is not influenced by propranolol (Fig 1).

Studies of serum gastrin concentrations during pharmacological stimulation or blockade of the β -adrenoceptors also have yielded conflicting results. Infusion of adrenaline at relatively high doses in healthy men causes a rise in serum gastrin (2,4) which is completely suppressed after pretreatment with the β -blocking agent pindolol (4). Interestingly, the gastrin response to adrenaline seems to be much more marked in patients with DU disease (24). Basal serum gastrin levels in both healthy subjects and ulcer patients are unchanged (5, 9, 25) or suppressed (6-7, 26, present study) by unselective β -blockade with propranolol. During insulin hypoglycemia gastrin levels are depressed by propranolol (5-7) although in one study, using a low dose of the drug (9), no effect on serum gastrin was found. In this study we show that β -blockade does not change serum gastrin concentration during stimulation with MSF and a previous study has demonstrated that alpha- and β -blockade cannot suppress the normal rise in serum gastrin in response to a meal (25).

In summary gastrin is released in response to high doses of β -agonists but whether the adrenergic nervous system can influence gastrin release under physiological conditions remains uncertain. One also has to take into consideration that the β -agonists might affect gastrin release indirectly via an influence on gastric acid secretion or mucosal blood flow.

The previous and present observations support the view that the sympathetic nervous system does not influence acid secretion during MSF, at least not in vagally intact individuals.

It has also been demonstrated recently that MSF, unlike insulin hypoglycemia, is not associated with alterations in plasma catecholamine concentrations (12). Apparently, MSF induces a more selective stimulation of the vagal innervation to the parietal cells than insulin.

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PIRENZEPINE DEPRESSES PLASMA NORADRENALINE IN DUODENAL ULCER PATIENTS

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SUMMARY

The effect of pirenzepine - a selective antimuscarinic compound - on plasma catecholamine, serum gastrin and somatostatin concentrations was studied in duodenal ulcer patients. Plasma concentration of noradrenaline decreased significantly after injection of pirenzepine (10 mg intravenously), whereas the circulating levels of adrenaline, dopamine, gastrin and somatostatin remained unchanged. The results may be explained by a decreased activity in postganglionic sympathetic neurones due to impaired transmission in sympathetic ganglia. This is in line with the recent observation in vitro that pirenzepine effectively blocks muscarinic receptors in some sympathetic ganglia.

Pirenzepine is the first antimuscarinic compound that can discriminate between different subclasses of muscarinic receptors (1). Accordingly, it is not surprising that pirenzepine markedly decreases basal and stimulated acid secretion without causing other antimuscarinic effects, except for a slight inhibition of salivation (2). It was recently shown by Hammer and colleagues (3,4) that pirenzepine also binds to a virtually uniform population of high affinity sites in sympathetic trunc ganglia and that it is about 50 times more potent in these ganglia than in the heart or in the smooth muscle of the upper gastrointestinal tract. Consequently, pirenzepine ought to block the transmission of nerve impulses in the sympathetic nervous system. Since plasma noradrenaline concentration is increased in patients with duodenal ulcer (5-7), the relevance of which is not known, it would be of interest to see whether pirenzepine can normalize plasma noradrenaline in this group of patients. The aim of the present study was therefore to investigate the effects of pirenzepine on circulating catecholamine, gastrin and somatostatin levels in patients with asymptomatic chronic duodenal ulcer disease.

Material and Methods

This investigation was approved by the Ethical Committee of the University of Lund and all patients gave their advised consent to participate in the study. The material consists of 4 males and 5 females (median age 46 years, range 33-68 years) with a history of peptic ulcer disease for more than one year. At least one duodenal ulceration had been verified by endoscopy in each case but at the time of this investigation they were without epigastric symptoms suggestive of active ulceration and free from medication.

All subjects were investigated in the sitting position the morning after an overnight fast and prohibition of smoking for 12 hours. A polyethylene catheter was implanted in an antecubital vein and at least 30 min elapsed between the introduction of the catheter and the first blood sample. Sampling was performed twice during basal conditions and then 10, 20, 30, 40, 50, 60, 90 and 120 min after an i.v. injection of 10 mg pirenzepine (Boehringer Ingelheim, FRG). Pulse rate and arterial blood pressure were recorded each time a blood sample was collected.

Plasma concentrations of adrenaline, noradrenaline and dopamine were measured using high pressure liquid chromatography with electrochemical detection (8). Serum gastrin (charcoal-separated plasma samples) and somatostatin concentrations were measured by radioimmunoassay as described elsewhere (9,10).

Data are expressed as median values and interquartile range. Wilcoxon's matched pairs signed-ranks test was used for calculation of statistical significance.

Results

No patient experienced discomfort during the experiments and no side effects of the drug were noticed. The pulse rate increased significantly ($p < 0.05$) from a basal value of 71 (70-72) beats/min to a maximum value of 76 (72-80) beats/min obtained 10 min after the injection of pirenzepine. The pulse rate then gradually returned to the basal level. No changes occurred in arterial blood pressure.

Pirenzepine caused a significant drop in plasma noradrenaline concentration (fig. 1) which fell from a median value of 1.65 nM to a nadir of 1.47 nM ($p < 0.05$) obtained 10 min after the injection of pirenzepine. Twenty min after the injection plasma noradrenaline was still significantly depressed (1.53 nM, $p < 0.02$) but it then gradually recovered towards the control value.

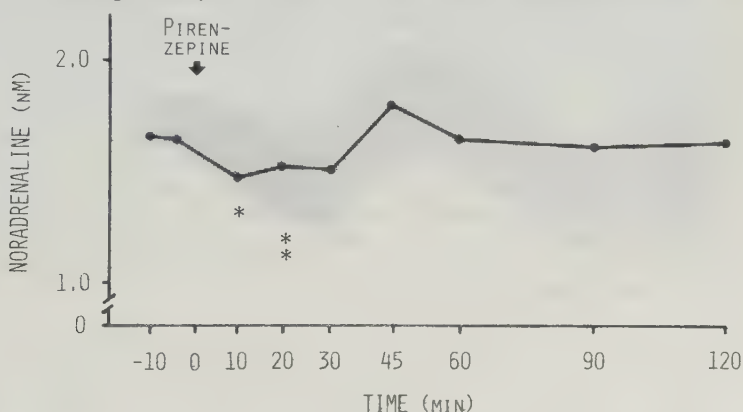


Fig 1

Plasma noradrenaline concentration before and after the i.v. injection of 10 mg of pirenzepine (arrow). Median values and interquartile ranges are shown. * = $p < 0.05$; ** = $p < 0.02$.

There were no significant alterations in plasma adrenaline (median value: 0.9 nM, range 0.7-0.11 nM) and dopamine (median value: 0.15 nM, range 0.12-0.22 nM) concentrations or in serum gastrin (median value: 20 pm/l, range 14-28 pm/l) and somatostatin (median value: 3 pm/l, range 2-5 pm/l) concentrations in response to pirenzepine.

Discussion

Several studies indicate that plasma catecholamines influence gastrin secretion both in healthy volunteers and duodenal ulcer (DU) patients (11-12). It has also been shown recently that plasma noradrenaline concentration is increased in DU patients (5-7) and normalized after highly selective vagotomy (13). However, it is still an unsettled question whether or not the elevated

plasma noradrenaline level has any etiological implication in DU disease. The present results, showing that pirenzepine decreased the elevated plasma noradrenaline levels in asymptomatic DU patients, indicate that these patients have an increased tone in their sympathetic nervous systems but the results do not reveal whether the hypernoradrenalinemia contributes to the disease or is merely a consequence thereof. The effect of pirenzepine on plasma noradrenaline, however, seems not mediated via its inhibitory action on gastric acid secretion (2) since acid reduction with cimetidine per se does not change plasma catecholamine concentrations (14).

Most likely, the depressive effect of pirenzepine on plasma noradrenaline is caused by interference with the ganglionic transmission of action potentials. Thus, in vitro studies have shown that pirenzepine has a 50-fold higher affinity for muscarinic receptors in sympathetic ganglia than in other tissues (3) and in vivo studies that amplification of the endogenous cholinergic activity results in increased plasma levels of noradrenaline, presumably due to stimulation of sympathetic postganglionic neurones (15). It is not likely that the decreased level of plasma noradrenaline is due to a direct release-inhibiting effect on the adrenergic nerve terminals. On the contrary, it has been shown that noradrenaline release can be inhibited by stimulation of presynaptic muscarinic receptors (16). Pirenzepine has no demonstrable effect on the postganglionic α_2 -receptors (Hammer, personal communication). A theoretical possibility that the decrease in plasma noradrenaline levels in response to pirenzepine is indirectly due to a reflex influence on the sympatho-adrenal system from cardiovascular receptors (17) is highly unlikely since no change in blood pressure and only a slight increase in pulse rate was observed.

Further support for the notion that the effect of pirenzepine in some way is linked to the sympathetic nervous system comes from a recent study on pylorus-ligated rats (18). Thus, the antisecretory effect of pirenzepine, but not of atropine, was markedly reduced when these rats were pretreated with 6-hydroxydopamine (known to destroy sympathetic neurons).

To our knowledge, the effect of other anticholinergic drugs on plasma catecholamine levels has not been studied. However, since muscarinic receptors in peripheral ganglia are almost exclusively of the high affinity type (3) there is no reason to believe that conventional unselective anticholinergic substances - compared to pirenzepine - would induce a greater change in plasma catecholamines.

The present results that pirenzepine did not change basal serum gastrin and somatostatin concentrations in man are corroborated by two previous studies (19,20). Evidently, the release of both these hormones under unstimulated circumstances is not controlled by the autonomic nervous system to any significant extent. The effect of pirenzepine on stimulated gastrin release, on the other hand, might be different (21) and there are data available suggesting that the gastrin response is attenuated by pirenzepine (22).

Acknowledgements

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EFFECTS OF UPPER ABDOMINAL SYMPHATHECTOMY ON GASTRIC ACID, SERUM GASTRIN AND CATECHOLAMINES IN THE RAT GUT

Short title: Gastric functions after sympathectomy

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ABSTRACT

Selective upper abdominal sympathectomy increased basal acid output in rats but was without effect on stimulated acid output, serum gastrin concentration and gastric mucosal histidine decarboxylase activity. The sympathectomy was verified by fluorescence histochemistry and determination of tissue catecholamines. A drastic reduction in tissue noradrenaline, adrenaline and dopamine levels occurred after sympathectomy, and fluorescence microscopy revealed a complete loss of adrenergic nerve fibers. Vagotomy reduced catecholamine levels in the stomach wall by 50 % but did not affect the catecholamine content in the pancreas and small bowel. Surprisingly, combined vagotomy and upper abdominal sympathectomy resulted in lower catecholamine levels than sympathectomy alone in extragastric but not in gastric tissues.

Key words: Acid secretion, adrenaline, dopamine, noradrenaline, gastrin, sympathectomy.

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INTRODUCTION

The distribution of adrenergic nerve fibers in the digestive tract has been documented by fluorescence histochemistry (1 - 3), but the role of the sympathetic nervous system in the control of gastric functions is poorly understood. Studies of sympathectomized animals should help to elucidate the functional significance of the adrenergic system with respect to the digestive tract. However, conflicting results have been obtained, possibly because many studies have employed chemical (4, 5, 6) or immunological (7) sympathectomy, procedures which eliminate sympathetic nerve fibers also outside the gastro-intestinal tract. Also pharmacological adrenoceptor blockade affects bodily functions in general. Studies using β -adrenoceptor blockers showed either no (8, 9) or slight increase (10) of basal acid output. Peak acid output after pentagastrin was reduced (9) or unaffected (8, 10) and insulin stimulation was less effective (11, 12). The varying results are probably due to different dosage of the drugs and the use of healthy volunteers or patients with ulcer disease. In a series of experiments in dogs Gottrup (13) found that β_2 -agonists reduced the acid output and the serum gastrin concentration. In man β -blockade reduced the serum gastrin concentration (14) or was without effect (15), whereas infusion of adrenaline increased serum gastrin (16, 17).

Selective abdominal sympathectomy should offer a better way to assess the role of the local sympathetic nerve supply in controlling digestive functions than total sympathectomy or adrenoceptor blockade. In previous studies upper abdominal sympathectomy had no (6, 18) or weak stimulatory effect on gastric acid secretion (4, 19, 20) and the serum gastrin concentration was found to increase (6).

The development of precise microsurgical techniques has made selective sympathectomy of the digestive tract possible. However, the results of the operation have to be verified chemically by measurement of catecholamines in the denervated tissues and histochemically by catecholamine histofluorescence. The aim of the present study was to analyze gastric acid secretion and to determine serum gastrin concentration after verified upper abdominal sympathectomy.

METHODS

Male Sprague Dawley rats, weighing approximately 200 g, were fed a standard diet of food pellets and tap water. One group ($n = 19$) was subjected to bilateral vagotomy as described by Shay et al (21). Pyloroplasty was performed at the same time. Upper abdominal sympathectomy was performed in 19 rats by the aid of an operation microscope. The procedure was essentially that of Holmes (22); the coeliac and superior mesenteric ganglia were extirpated and the coeliac branch of the posterior vagal trunk was cut. One group ($n = 16$) was subjected to both vagotomy and upper abdominal sympathectomy. Gastric fistula rats ($n = 10$) were prepared as described in (23). Operated rats were left for at least four weeks before sacrifice after 48 hours without food (free access to water). Fistula rats were fasted for 24 hours before studies of gastric secretion. Pylorus ligation ($n = 8$) was performed by laparotomy, using

a midline incision of approximately 3 cm under light diethyl ether anesthesia. The pyloric portion of the stomach was gently mobilized and occluded with a 4-0 silk ligature around the sphincter in the manner described by Shay et al (24). The rats were killed 4 hours later. In acid secretory studies using conscious gastric fistula rats all animals were restrained in Bollman-type cages. 10 ml 0.9 % saline were given s.c. and the cannulas were opened and allowed to drain freely for 1 h. Basal acid secretion was then collected in two one-hour portions. The gastric juice was collected for 4 hours after the administration of maximal or near-maximal doses of pentagastrin (Peptavlon ICI) 250 µg/kg s.c. or histamine dihydrochloride 25 mg/kg s.c. Acid output was determined by titration with 0.02 N NaOH, using phenolphthalein as indicator. The animals were bled to death under ether anesthesia by collecting blood from the aorta. Serum samples were stored at -20° C. The stomach was dissected out and the antral and acid producing parts were separated. The mucosa was scraped off the muscle layer and collected separately for determination of catecholamines and (in the case of the oxyntic mucosa) histidine decarboxylase activity. Also, the pancreas, upper part of the duodenum and middle portion of the small bowel were collected together with the spleen for determination of catecholamines. The samples were weighed and placed in 4 % perchloric acid, homogenized and filtered. Adrenaline, noradrenaline and dopamine were determined by high performance liquid chromatography (25). Histidine decarboxylase activity in the gastric mucosa was determined radiometrically as described earlier (26) and serum gastrin was determined by radioimmunoassay (courtesy Dr J Rehfeld) using antiserum 2604 (27). The Mann-Whitney U-test was used for assessment of statistical differences. Tissue specimens were processed also for the fluorescence histochemical demonstration of biogenic amines (28). Specimens were frozen in propane-propylene to the temperature of liquid nitrogen. After freeze-drying they were exposed to formaldehyde gas for 1 h at 80° C according to the standard Falck-Hillarp technique (28) and embedded in paraffin. Sections were cut at 8 µm thickness, deparaffinized and mounted in Entellan^R. The sections were examined in a fluorescence microscope (exciting wavelength 405 nm).

RESULTS

Operated animals recovered well and the mortality after upper abdominal sympathectomy was below 10 per cent. After sympathectomy the rats often had a transient period (4 - 5 days) of diarrhoea. The animals were killed 4 - 5 weeks after surgery. As seen in Table I the noradrenaline concentration in unoperated animals was quite high in the spleen and in the acid producing part of the stomach wall. After sympathectomy the noradrenaline values were reduced to 10 or less than 10 per cent of the predenervation levels in all tissues studied. Truncal vagotomy reduced the noradrenaline concentration in the stomach wall by about 50 per cent; the concentration of noradrenaline in the duodenum, midgut, pancreas and spleen was unaffected. The combined procedure (vagotomy and sympathectomy) resulted in significantly lower noradrenaline concentrations in extragastric but not in gastric tissue than sympathectomy alone. Adrenaline and dopamine concentrations (Table II and III) changed in parallel with noradrenaline. Fluorescence histochemistry revealed an absence of adrenergic nerve fibers in the pancreas, spleen and upper digestive tract after sympathectomy.

Acid secretory tests using fistula rats showed an increase in basal acid output following sympathectomy (131 ± 13 $\mu\text{Eq H}^+/\text{h}$ compared to 59 ± 16 in control rats, $p < 0.01$). The peak acid output after pentagastrin (Fig. 1) or histamine (Fig. 2) was slightly increased (not statistically significant). Acid secretion after pylorus ligation showed the same tendency (734 ± 38 $\mu\text{Eq H}^+/\text{4 hr}$ compared to 500 ± 64 in control rats). The basal gastric histidine decarboxylase activity was unaffected by sympathectomy (6.1 ± 1.1 pmoles $\text{CO}_2/\text{mg.hr}$ compared to 5.8 ± 0.4 in control rats) and serum gastrin levels in sympathectomized rats (25 ± 3 pg/ml) did not differ from normal values (26 ± 4).

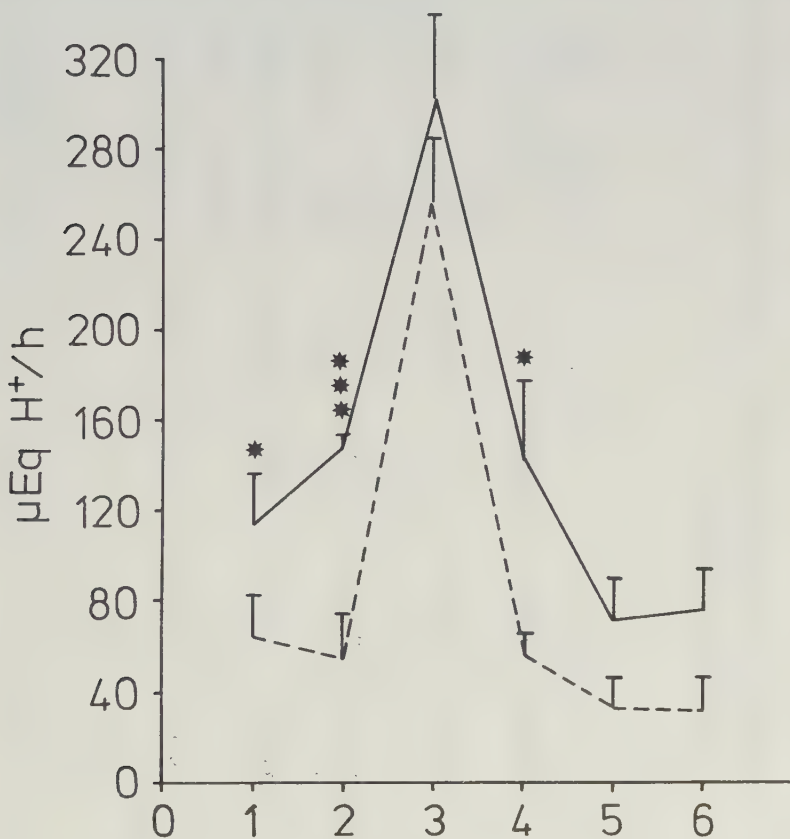


Fig. 1

Acid output ($\mu\text{Eq h}^{-1}$) in response to pentagastrin ($250 \mu\text{g/kg b.w.}$) in sympathectomized (solid line) and control (broken line) rats. $n = 8$ in each group. Mean $\pm$ S.E.M. * $p < 0.05$. *** $p < 0.01$

TABLE I Noradrenaline concentration (ng/g) in various tissues after different denervation procedures.

Denervation	Fundus		Antrum		Duodenum	Midgut	Pancreas	Spleen
	mucosa	muscle	mucosa	muscle	whole wall			
Control (n = 20)	274 (21) +++	1 143 (86) +++	471 (29) +++	668 (60) ++	696 (75) +++	615 (33) +++	927 (46) +++	1 283 (64) +++
Sympathectomy (n = 19)	6.6 (1.7) +++	40.2 (7.4) ++	15.4 (3.3) +++	24.6 (5.6) +++	44.5 (2) n.s.	46 (3.6) n.s.	94 (8) n.s.	38.6 (6) n.s.
Vagotomy (n = 19)	114 (16) +++	334 (31) +++	207 (24) +++	331 (12) +++	863 (33) +++	639 (23) +++	726 (29) +++	1 260 (39) +++
Vagotomy and sympathectomy (n = 16)	26.1 (4.8) +++	13.8 (1.9) +++	21.4 (1.1) +++	28 (3.2) +++	3.1 (1.5) +++	1.4 (0.2) +++	6.1 (1.1) +++	5.1 (0.7) +++

Mean ($\pm$ SEM). + <0.05 ++ <0.02 +++ <0.01. n.s. = not significant compared to control rats

TABLE II Adrenaline concentration (ng/g) in various tissues after different denervation procedures.

Denervation	Fundus		Antrum		Duodenum whole wall	Midgut	Pancreas	Spleen
	mucosa	muscle	mucosa	muscle				
Control (n = 20)	16.6 (1.5) +++	30.1 (2.1) +++	55.7 (7) +++	46 (4) +++	21 (2) +++	15 (2) +++	22 (3) +++	43.8 (2.8) +++
Sympathectomy (n = 19)	2.3 (0.2) ++	7.2 (1.2) ++	7.9 (1.1) ++	4.9 (0.9) +	10 (1.5) n.s.	7.7 (0.6) +	12 (2) n.s.	1.2 (0.1) n.s.
Vagotomy (n = 19)	11.4 (1.8) +++	10.7 (1.5) +++	18 (3.1) +++	21 (3.6) +++	18 (1.8) +++	9.4 (0.7) +++	21.8 (3.6) +++	50.8 (2.2) +++
Vagotomy and sympathectomy (n = 16)	3.6 (0.2) +++	5.1 (0.8) +++	10.4 (1.7) +++	5.4 (0.2) +++	7.4 (0.7) +++	3.4 (0.9) +++	2.9 (0.2) +++	1.9 (0.4) +++

Mean ($\pm$ SEM). + ≤ 0.05 ++ ≤ 0.02 +++ ≤ 0.01 . n.s. = not significant compared to control rats

TABLE III Dopamine concentration (ng/g) in various tissues after different denervation procedures.

Denervation	Fundus		Antrum		Duodenum	Midgut	Pancreas	Spleen
	mucosa	muscle	mucosa	muscle	whole wall			
Control (n = 20)	31.5 (2) +++	35.2 (2) +++	27 (3) +++	25 (2.3) +++	19.6 (1.9) +++	16 (1.7) +++	23.5 (1.8) ++	32.9 (2.8) +++
Sympathectomy (n = 19)	8.2(0.5) +++	3.1 (0.3) +++	9 (2) +	7.1 (1) n.s.	5 (0.9) n.s.	5.1 (0.6) n.s.	6.7 (0.7) n.s.	2.4 (0.4) n.s.
Vagotomy (n = 19)	17.1(1.5) +++	12.7 (1.4) +++	18 (3.7) +++	18 (2+3) ++	20.6 (2.5) +++	19 (1.2) +++	21.8 (2.7) +++	36.4 (2.5) +++
Vagotomy and sympathectomy (n = 16)	14.8(0.6) +++	5.3 (0.2) +++	12.7 (1.1) +++	10.9 (1.8) ++	5.3 (0.3) +++	6.6 (0.5) +++	3.5 (0.9) +++	2.1 (0.4) +++

Mean ($\pm$ SEM). + < 0.05 ++ < 0.02 +++ < 0.01. n.s. = not significant compared to control rats.

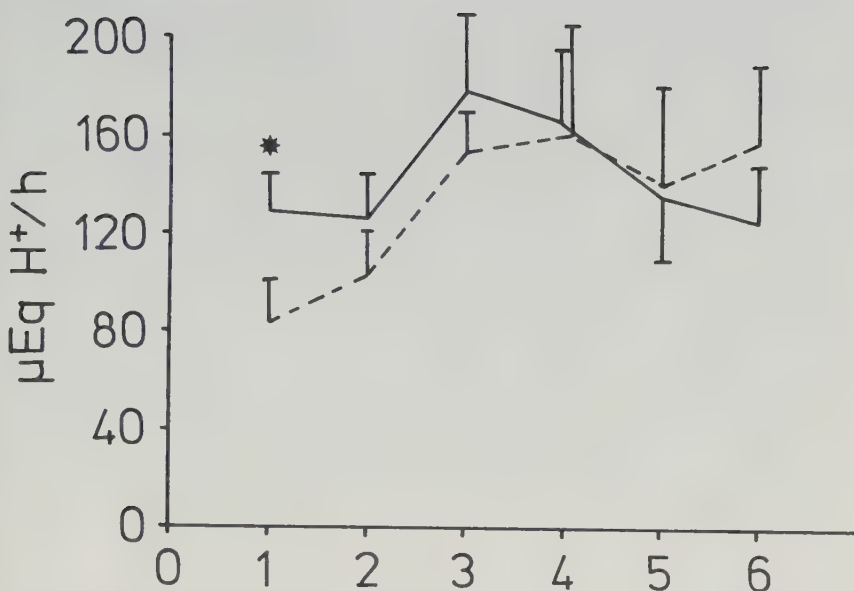


Fig. 2 Acid output ($\mu\text{Eq}/\text{h}$) in response to histamine (25 mg/kg b.w.) in sympathectomized (solid line) and control (broken line) rats. $n = 8$ in each group. Mean $\pm$ S.E.M. * $p < 0.05$

DISCUSSION

Studies on the role of the sympathetic nervous system in the control of upper gastrointestinal functions have provided conflicting results, the reason possibly being that pharmacological stimulation or blockade of adrenoceptors influence sympathetic neurotransmission in general. The same applies to chemical and immunological sympathectomy. Selective upper abdominal sympathectomy appears to offer several advantages over alternative procedures. However, the completeness of surgical sympathectomy depends upon the technique and must be verified chemically and histochemically. The present study was carried out on rats subjected to upper abdominal sympathectomy using a microsurgical approach and the resulting denervation was assessed by measuring the catecholamine levels and by fluorescence microscopic examination of the tissues.

Most noradrenergic nerve terminals were seen to ramify around the neurones of the myenteric and submucous ganglia (3). The highest catecholamine concentrations in the upper gastrointestinal tract of unoperated rats were found in the muscle layer of the acid producing part of the stomach. The catecholamine concentration of the stomach wall was reduced not only by sympathectomy but also by vagotomy, probably because the vagi contain adrenergic fibers (29). Ahlman et al (30) have suggested that the adrenergic fibers in the vagus derive from the stellate and superior cervical ganglia. Since vagotomy did not reduce catecholamine levels in other tissues the results suggest that the adrenergic fibers in the vagus supply the stomach wall exclusively. Surprisingly, however, the combined procedure, sympathectomy and vagotomy, led to reduced catecholamine concentrations - compared to sympathectomy alone -in extragastric but not in gastric tissue. The reason for this paradoxical observation and the reason why catecholamine concentrations did not drop to zero after denervation are obscure. It is unlikely that catecholamines in blood can explain the residual concentrations found in sympathectomized tissue. Another explanation is the possible existence of intramural adrenergic ganglia which would not be affected by the surgical procedure used in this study. However the results of fluorescence microscopic examination indicated a complete loss of adrenergic neuronal elements in the gut and pancreas after sympathectomy. Hence, the fact that some dopamine, noradrenaline and adrenaline remained after sympathectomy cannot be explained at present.

Chemical sympathectomy has been found to increase gastric acid secretion (4, 5, 6) while surgical sympathectomy has no or very slight influence on acid output (4, 6, 18, 31). The latter finding was confirmed in the present study. Chemical sympathectomy provides complete sympathectomy but denervates also organs other than the digestive tract. This could explain the different acid output after surgical compared with chemical sympathectomy. In the present study only a slight change in basal acid secretion was observed and gastric mucosal histidine decarboxylase activity and serum gastrin levels did not differ from controls. In summary, upper abdominal sympathectomy in the rat drastically reduced the catecholamine content in the denervated tissues with no or very slight changes in acid secretion, gastrin release and gastric histidine decarboxylase activity. In contrast vagotomy, known to have major effects on acid secretion and gastrin release, reduced catecholamine levels in the stomach wall by 50 %. Peptide-containing nerves, constituting a prominent part of the enteric nervous system, are predominantly of intrinsic origin (32). Of particular interest with respect to the sympathetic control of digestive functions is the fact that the newly discovered neuropeptide NPY(33) coexists with noradrenaline in a population of sympathetic nerve fibers (34). The physiological role of NPY is not known.

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PEPTIDE-CONTAINING NERVE FIBERS IN THE STOMACH WALL OF RAT AND MOUSE

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Short title: Peptide-containing nerve fibers in stomach

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ABSTRACT

Peptide-containing nerve fibers were numerous in the glandular stomach of the rat and mouse. The immunoreactive neuropeptides demonstrated included vasoactive intestinal polypeptide (VIP), gastrin-releasing peptide (GRP), substance P (SP), enkephalin, somatostatin, gastrin/cholecystokinin (CCK) and NPY. Each system of peptide-containing nerve fibers had its characteristic topographic distribution. On the whole the topography and frequency of the different types of peptide-containing nerve fibers were fairly similar in the acid producing and prepyloric parts of the stomach. The mucosa harboured numerous VIP and GRP fibers, and a modest number of SP and NPY immunoreactive fibers. Mucosal nerve fibers were found in the lamina propria, sometimes extending high up between the glands. In the submucosa they were found around blood vessels and in the submucosal ganglia. Blood vessels received a particularly rich supply of NPY fibers. The smooth muscle and the myenteric ganglia harboured not only VIP, GRP, SP and NPY-immunoreactive fibers but also enkephalin, somatostatin and gastrin/CCK-immunoreactive fibers. VIP, GRP, SP, enkephalin and NPY-immunoreactive nerve cell bodies could be detected in the myenteric ganglia; they appeared somewhat more numerous and more intensely immunoreactive after local or systemic colchicine treatment. Vagal denervation did not affect the density and distribution of the various peptide-containing nerve fibers. Following sympathectomy mucosal and perivascular NPY fibers disappeared. The other types of peptide-containing nerve fibers were not affected.

INTRODUCTION

Nervous mechanisms are important for the regulation of gastric functions. The central neural control is mediated by vagal (parasympathetic) and splanchnic (sympathetic) nerves. In addition, intramural neuronal systems exert local control of functional activity. Recent observations suggest that peptide-containing nerve fibers represent a quantitatively prominent part of the nerve supply of the stomach (1-6). Such fibers may derive from extrinsic (extramural) or intrinsic (intramural) sources. The present paper describes the occurrence, distribution and origin of nerve fibers that can be demonstrated histochemically with antisera against vasoactive intestinal polypeptide (VIP), substance P (SP) somatostatin, gastrin/cholecystokinin (CCK), enkephalins, gastrin-releasing peptide (GRP) or NPY (7) in the stomach of the rat and mouse.

MATERIALS AND METHODS

Sprague-Dawley rats and NMRI mice of both sexes were used. Five rats were subjected to bilateral subdiaphragmatic truncal vagal denervation and pyloroplasty (8,9). Upper abdominal sympathectomy was performed on 4 rats by extirpating the coeliac and superior mesenteric ganglia; this procedure also sectioned the coeliac branch of the posterior vagus trunk. Operated rats were left for 3-4 weeks before sacrifice. In 2 of the sympathectomized animals the completeness of the sympathectomy was verified chemically by determination

of norepinephrine (10) in the gut wall (courtesy of Prof. E. Rosengren, Department of Pharmacology, University of Lund, Lund, Sweden). Such determinations revealed a more than 90 % reduction of the norepinephrine content in the gastric wall (assayed in 2 controls and 2 denervated rats) (see also ref. 11). Two mice and 2 rats were given colchicine (BDH, Poole, England) (10 mg/kg) intraperitoneally and killed 24 hours later. Two rats were given colchicine (20 μ g) by local injection into the wall of the acid-secreting part of the stomach. Some of the animals were anesthetized with diethyl ether and perfused via the heart with an ice-cold solution of 4 % formaldehyde in 0.1 M phosphate buffer, pH 7.2, for 5 min. Specimens from the oxyntic gland area and the pyloric gland area were immersed in the fixative for 10-12 hours and thoroughly rinsed in buffer enriched with 5-25 % sucrose. Alternatively, the animals were killed, the tissue specimens dissected out and fixed by immersion in ice-cold 4 % formaldehyde solution over-night. After thorough rinsing, perfusion- as well as immersion-fixed specimens were frozen on dry ice and sectioned in a cryostat at 10-20 μ m thickness. Whole mounts were prepared by stretching a 1x1 cm segment of the stomach wall on a piece of balsa wood with the mucosa facing the wood; the preparation was left floating on a mixture of formalin-picric acid (Stefanini's fluid) for 12-24 hours followed by rinsing in buffer containing 25 % sucrose. The smooth muscle layers were separated from each other and from the mucosa-submucosa with a pair of forceps and placed on slides where they were allowed to dry followed by freezing and thawing. Sections and whole mounts were processed for the immunocytochemical demonstration of gastrin/CCK, GRP, VIP, SP and somatostatin using previously characterized rabbit antisera (Table 1). Neuropeptide Y (NPY)-like peptides (18) were demonstrated using an antiserum raised against porcine NPY and an antiserum raised against avian pancreatic polypeptide (APP) (7). The APP antiserum cross-reacted with NPY (see also 6). The NPY antiserum did not cross react with APP (6). The immunoreactive material demonstrated by these two antisera in neuronal elements is referred to as NPY. The site of the antigen-antibody reaction was visualized by the indirect immunofluorescence method of Coons et al. (19).

For controls we used antisera which had been inactivated by the addition of excess amounts of pure antigen (10-100 μ g/ml). APP was provided by Professor J.R. Kimmel, University of Kansas, Kansas City, Kansas, USA. Porcine VIP and NPY (6) were obtained from Professor V. Mutt and Dr. K. Tatemoto respectively, both at Karolinska Institute, Stockholm, Sweden. CCK-8 (Sincalid^(R)) was generously provided by Squibb, New Brunswick, N.J., USA. Substance P, Leu- and Met-enkephalin and somatostatin were provided by Peninsula, Calif., USA. Cross-reaction with other peptides or proteins containing amino acid sequences recognized by the different antisera cannot be excluded. It is appropriate therefore to refer to the immunoreactive material as gastrin/CCK-like, GRP-like, VIP-like and so on. For simplicity, however, the shorter terms are used in the following.

TABLE 1 Details of the antisera

Antigen	Code	Raised against	Directed against	Immu- fluoresc.	Working dilution PAP- staining	Source	Ref
VIP	7852	unconj. porcine VIP		1:160	1:5120	Milab, Malmö, Sweden	12
GRP ¹⁾	6902	protein-conj. porcine GRP	C-terminus	1:640	1:10240	N. Yanaihara, Shizuoka, Japan	13
Substance P ²⁾	SV8	protein-conj. bovine substance P	C-terminus	1:40	1:320	P.C. Emson, Cambridge, England	14
Met-enkephalin ³⁾	Met-enk	protein-conj. Met-enkephalin	C-terminus	1:80	1:640	R.J. Miller and K.J. Chang, Burroughs Wellcome Research Triangle Park, N.J. USA	15
Leu-enkephalin ³⁾	Leu-enk	protein-conj. Leu-enkephalin	C-terminus	1:80	1:240	" "	15
Somatostatin	19578	protein-conj. ovine somatostatin	-	1:160	1:2560	M.P. Dubois, Nouzilly, France	16
	K18	- " -	-	1:100	1:1600	Milab, Malmö Sweden	-
Gastrin 2-17 ⁴⁾	4562	protein-conj. human gastrin 2-17	C-terminus	1:640	1:5120	J.F. Rehfeld, Copenhagen, Denmark	17
APP ⁵⁾	APP 123	unconj. chicken PP	Mid portion or N-terminus	1:80	1:640	J.R. Kimmel Univ. of Kansas, Kansas City, Kansas, USA	7
NPY ⁵⁾		unconj. porcine NPY		1:400		P.C. Emson, MRC Cambridge, England	6

1) Cross-reaction with bombesin at the immunocytochemical level.

2) No cross-reaction with bombesin and GRP.

3) The Leu-enkephalin antiserum cross-reacts with Met-enkephalin and the Met-enkephalin antiserum with Leu-enkephalin.

4) Cross-reaction with cholecystokinins that share the C-terminus pentapeptide with gastrin

5. The NPY antiserum does not cross-react with APP. The APP antiserum cross-reacts with NPY.

RESULTS

All the antisera used demonstrated neuronal elements in the gastric wall. Nerve fibers displaying VIP, SP, GRP or NPY immunoreactivity had a widespread distribution in the gastric wall of both rat and mouse; they occurred in the mucosa, submucosa and muscle coat, including the myenteric ganglia. Such nerve fibers were seen also around blood vessels, particularly in the submucosa and submucous ganglia; in the latter location peptide-containing nerve cell bodies could be demonstrated. The distribution of peptide-containing nerve fibers was studied in both the pyloric and the oxyntic gland area. No regional difference in nerve fiber density and topography or in number and distribution of the various types of nerve cell bodies could be revealed. VIP and GRP fibers were quite numerous in the mucosa. SP, enkephalin and NPY fibers were few in the mucosa but somewhat more numerous in the submucosa. Somatostatin and gastrin/CCK fibers were absent altogether from the mucosa and submucosa. VIP, GRP, SP, enkephalin and NPY fibers were numerous in the smooth muscle and the myenteric ganglia. Somatostatin and gastrin/CCK fibers were few and confined to the smooth muscle and myenteric ganglia. Immunoreactive nerve cell bodies occurred in the myenteric ganglia only. VIP, SP and NPY cell bodies could be detected without colchicine pretreatment, whereas colchicine was required to reveal GRP and enkephalin nerve cell bodies. VIP cell bodies were particularly numerous, whereas GRP, SP, enkephalin and NPY cell bodies were much fewer. No somatostatin and gastrin/CCK cell bodies could be detected. The results were quite similar in the two species examined. The findings are summarized in Table II and Fig. 1.

Distribution of individual types of peptide-containing nerve fibers

VIP. Numerous fine-varicose, moderately immunoreactive fibers were seen to run beneath and around the glands as well as in the submucosa in both the oxyntic and the pyloric gland areas (Fig. 2a). Although the nerve fibers predominated basally in the mucosa, many fibers could be seen to reach high up between the oxyntic glands (Fig. 2a). The submucous ganglia contained scattered immunoreactive nerve fibers. In the smooth muscle layers and in the myenteric ganglia the VIP fibers were more coarse and more intensely immunoreactive. The inner half of the smooth muscle layer was more densely innervated than the outer layer. Generally, the fibers were seen to run along the smooth muscle bundles. The myenteric ganglia were densely innervated. Here the fibers were more coarse and more intensely immunoreactive than in the smooth muscle. They formed a dense network around immunoreactive as well as non-immunoreactive nerve cell bodies. VIP cell bodies were lacking in the submucous ganglia but were regularly observed in the myenteric ganglia; they appeared more intensely immunoreactive after local or systemic colchicine treatment.

GRP. Fine-varicose fibers formed a dense network in the mucosa and submucosa (Fig. 2b and c), including the submucous ganglia. Numerous mucosal GRP fibers extended all the way up to the mucosal surface (Fig. 2b and c). GRP fibers were numerous also in the smooth muscle and in the

myenteric ganglia. They were more numerous in the inner half of the smooth muscle layer than in the outer half. In the smooth muscle and myenteric ganglia the fibers were generally more coarse and more intensely immunoreactive than in the mucosa (Fig. 3 a-c). A moderate number of GRP immunoreactive nerve cell bodies was seen in the myenteric ganglia after colchicine treatment (Fig. 2d). GRP fibers were seen to surround immunoreactive as well as non-immunoreactive nerve cell bodies.

SP. SP fibers were few in the mucosa and submucosa and appeared as delicate, varicose fibers extending up to the mid portion of the oxyntic and pyloric glands. Scattered fibers were seen in the submucous ganglia. In the smooth muscle and in the myenteric ganglia SP fibers were fairly numerous and more intensely immunoreactive than in the mucosa (Fig. 2e). Like VIP and GRP fibers, SP fibers were more numerous in the circular smooth muscle layer than in the longitudinal. An occasional immunoreactive cell body could be detected within the myenteric ganglia; they were lacking in the submucous ganglia. Treatment with colchicine had no clear-cut effect on the number of immunoreactive cell bodies.

Enkephalin. Enkephalin fibers were few in the mucosa, submucosa and submucous ganglia. At most one or two fibers could be detected in each section. In contrast, enkephalin fibers were fairly numerous in the myenteric ganglia and smooth muscle, particularly in the circular muscle layer. Occasionally immunoreactive cell bodies were seen in the myenteric ganglia, especially after colchicine treatment (Fig. 4a). The two antisera (raised against Leu-enkephalin and Met-enkephalin, respectively) gave indistinguishable results.

Somatostatin. Somatostatin fibers could be demonstrated in low numbers in the smooth muscle and in moderate numbers in the myenteric ganglia. The mucosa and submucosa seemed to lack somatostatin fibers altogether. No immunoreactive nerve cell bodies could be demonstrated in either the myenteric or the submucous ganglia, not even after colchicine treatment. Somatostatin-containing endocrine/paracrine cells occurred scattered in the epithelium throughout the glandular stomach in both species as previously described (20).

Gastrin/CCK. Immunoreactive fibers were few in the smooth muscle and in the myenteric ganglia (Fig. 4d). None was seen in the mucosa or the submucous ganglia. Immunoreactive nerve cell bodies could not be detected in either the myenteric or the submucous ganglia, not even after colchicine. Immunoreactive endocrine cells, identical with gastrin cells, were seen in the antrum mucosa.

NPY. Similar histochemical pictures were obtained with the NPY and the APP antisera. Fairly numerous fibers were seen in the smooth muscle but they were quite few in the myenteric ganglia (Fig. 4b-c). NPY fibers were fairly numerous also in the basal part of the mucosa and in the submucosa, notably around blood vessels (small arteries). Single fibers were seen running close to the glandular base. The submucous ganglia contained fairly many NPY fibers. An occasional immunoreactive nerve cell body was seen in the myenteric ganglia (Fig. 4b). Occasionally, a few immunoreactive endocrine cells were seen scattered in the rat oxyntic mucosa.

Denervation experiments

Vagotomy. Vagal denervation did not visibly affect the density and distribution of the various peptide-containing nerve fibers.

Sympathectomy. The rich supply of NPY nerve fibers in the smooth muscle seemed unaffected by the sympathectomy whereas mucosal NPY fibers had disappeared. Also the vascular NPY nerve supply had disappeared. The results were the same with the APP and the NPY antisera. The other types of peptide-containing nerve fibers were not affected by the sympathectomy.

TABLE II

Topographic distribution and relative frequency of peptide-containing nerve fibers in the stomach wall of the rat and mouse. (No difference between oxyntic and pyloric areas).

Immunoreactive peptide	Mucosa and submucosa	Smooth muscle	Myenteric ganglia
VIP	+++	+++	+++
CRP	+++	+++	+++
SP	+	++	++
Enkephalin	+	++	++
Somatostatin	0	+	+
Gastrin/CCK	0	+	+
NFP	++	++	+

+++ numerous fibers

++ moderate number

+ few

0 no fibers detected

The estimates reflect assessments made by two independent observers and are based on examination of 5 to 10 specimens (at least two sections from each specimen). Specimens from 5 rats and 5 mice were analyzed.

DISCUSSION

The nervous control of gastric functions is still poorly understood. It is generally agreed that the central nervous system exerts a strong influence via the vagus nerve in particular, but it is now recognized that in addition intramural neuronal systems play an important part. The role of conventional neurotransmitters, such as acetylcholine and norepinephrine has been explored using specific receptor agonists and antagonists. During the course of such studies it has become increasingly apparent that all neuronally mediated responses in the digestive tract cannot be explained by the release of acetylcholine or norepinephrine (for references see 21-22). Recently, interest has focused on a new group of putative transmitters, the neuropeptides, which occur in great number throughout the gut wall (for references see 23-25). Their distribution and functional significance have been studied extensively in the intestines, whereas much less information is available on the stomach (1-6). The present study was performed in order to outline the anatomy of the various systems of peptide-containing nerve fibers in the stomach of the rat and mouse, thereby providing a basis for future functional studies. Many of the neuropeptides have potent effects on gastric functions although information is far from complete (for references see 26-30).

On the whole, the present findings on the distribution of the various peptide-containing neuronal systems in the stomach confirm earlier results with respect to SP, VIP, enkephalin, somatostatin and gastrin/CCK (2), GRP (4,5) and NPY (6). Minor differences from earlier descriptions may be noted. Unlike us, Buffa et al. (5) described a more dense GRP nerve supply in the antrum than in the acid-producing part of the stomach, and we failed to detect mucosal gastrin/CCK fibers but demonstrated somatostatin fibers in contrast to a previous report (2).

In view of the fact that VIP and GRP fibers were conspicuously numerous in the mucosa it is tempting to suggest that they regulate important functions in this location. This may be the case also for SP, enkephalin and NPY fibers although they were much fewer. Somatostatin and gastrin/CCK fibers, however, were absent from the gastric mucosa. All neuropeptides recognized in the gastric mucosa so far seem to suppress acid secretion (Ekelund et al., to be published). The exact nature of the neuropeptidergic influence on mucosal functions largely remains to be elucidated. While somatostatin and gastrin/CCK fibers were few, the smooth muscle of the stomach wall received a conspicuously rich supply of VIP, GRP, SP, enkephalin and NPY fibers. This may suggest an important role for these peptides in the control of gastric motor activity.

The following observations suggest a predominantly intrinsic origin of peptide-containing nerve fibers in the stomach wall: Nerve cell bodies containing immunoreactive VIP, GRP, SP, enkephalin or NPY were detected in the intramural ganglia. Vagal denervation did not visibly affect the number and distribution of peptide-containing nerve fibers. Also sympathetic denervation was without effect except that NPY fibers around blood vessels and in the mucosa and submucosa disappeared, suggesting an extrinsic source of a

significant proportion of the NPY nerve supply. It has been suggested that norepinephrine and NPY co-exist in sympathetic nerve fibers (31-34). The NPY fibers that remain after sympathectomy in the smooth muscle of the stomach are clearly not norepinephrine-containing since the norepinephrine-containing fibers in the stomach wall are sympathetic in origin (35). Conceivably therefore, NPY fibers are of two different types, those that contain norepinephrine and those that do not.

Although previous studies combining immunocytochemistry and immunochemical analyses have revealed the presence of neuronal VIP (36), SP (14,37,38), somatostatin (39), CCK-8 (40,41), Leu- and Met-enkephalin (42), neurotensin and GRP (4,13,43) in the intestines of several species, it should be noted that the present results on the stomach are from immunocytochemical studies exclusively, which means that the exact identity of the immunoreactive material remains to be established. The present findings confirm and extend previous immunocytochemical observations (1-6).

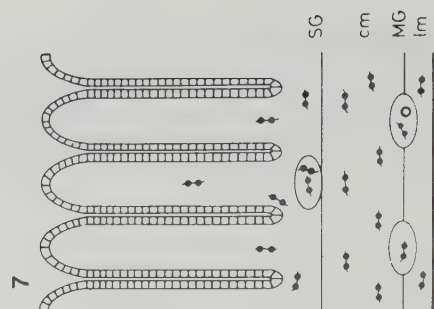
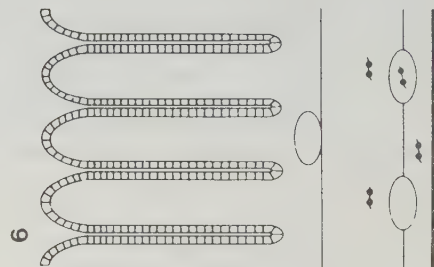
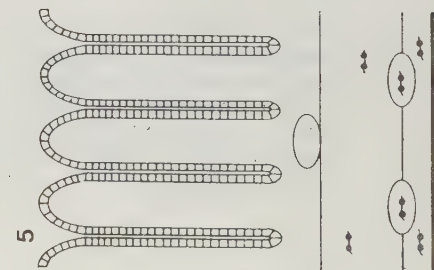
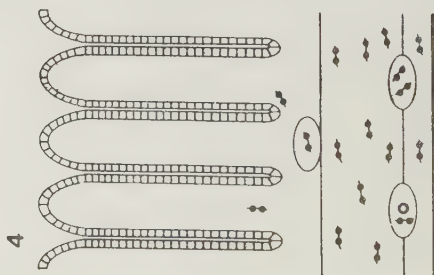
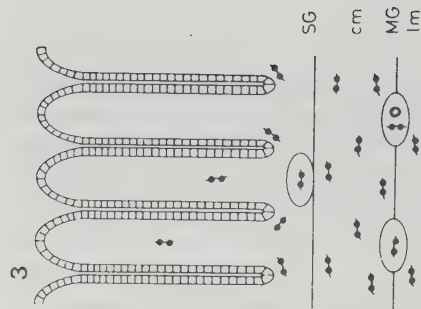
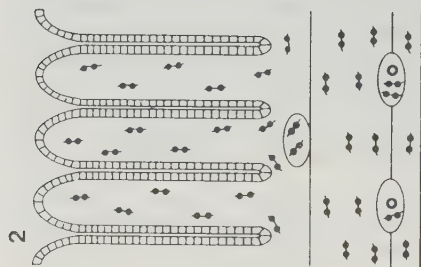
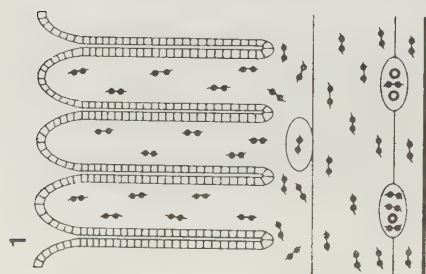
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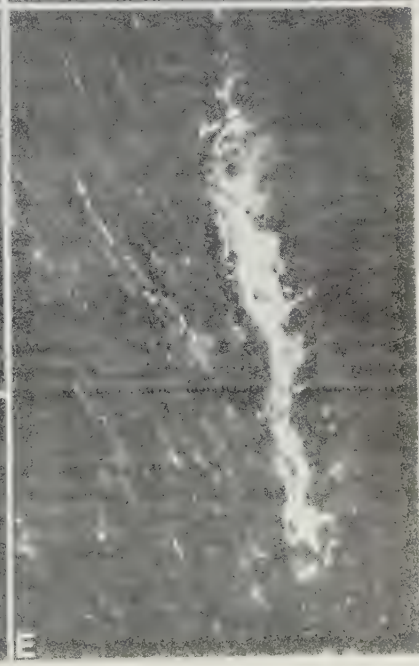
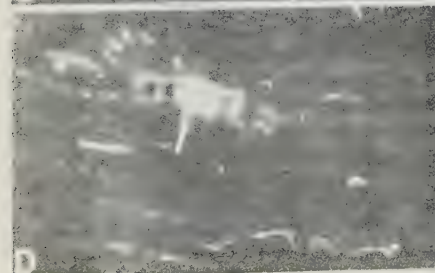
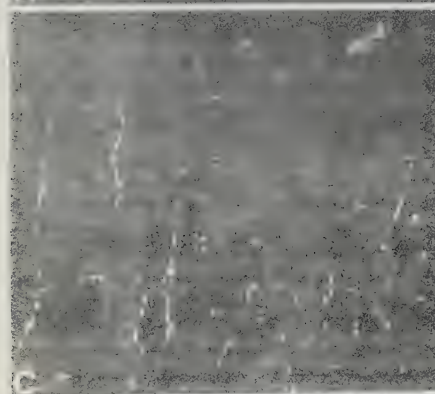
Grant support from the Swedish MRS (14X-4499, 04X-1007) and A. Pahlsson's Foundation.

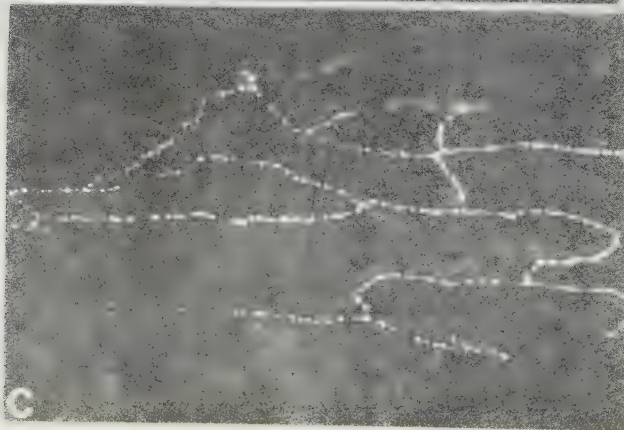
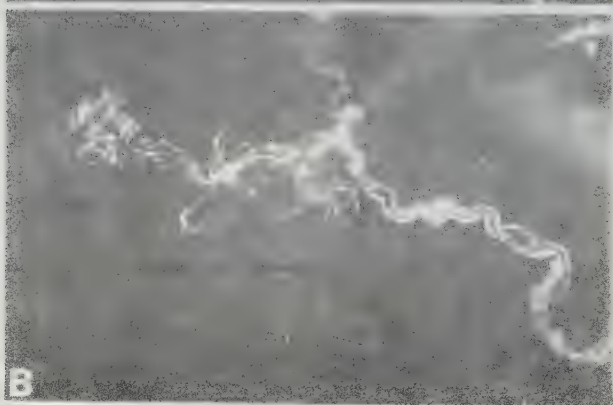
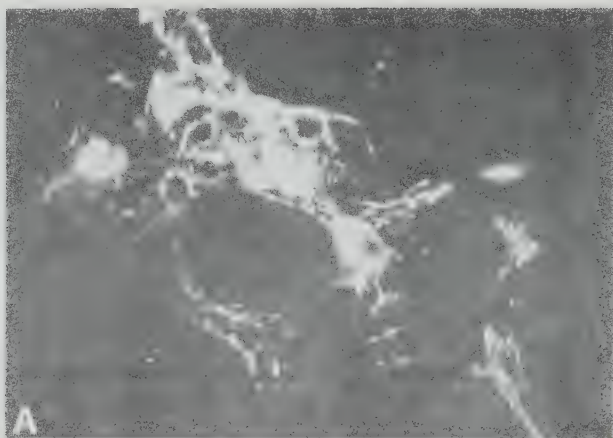
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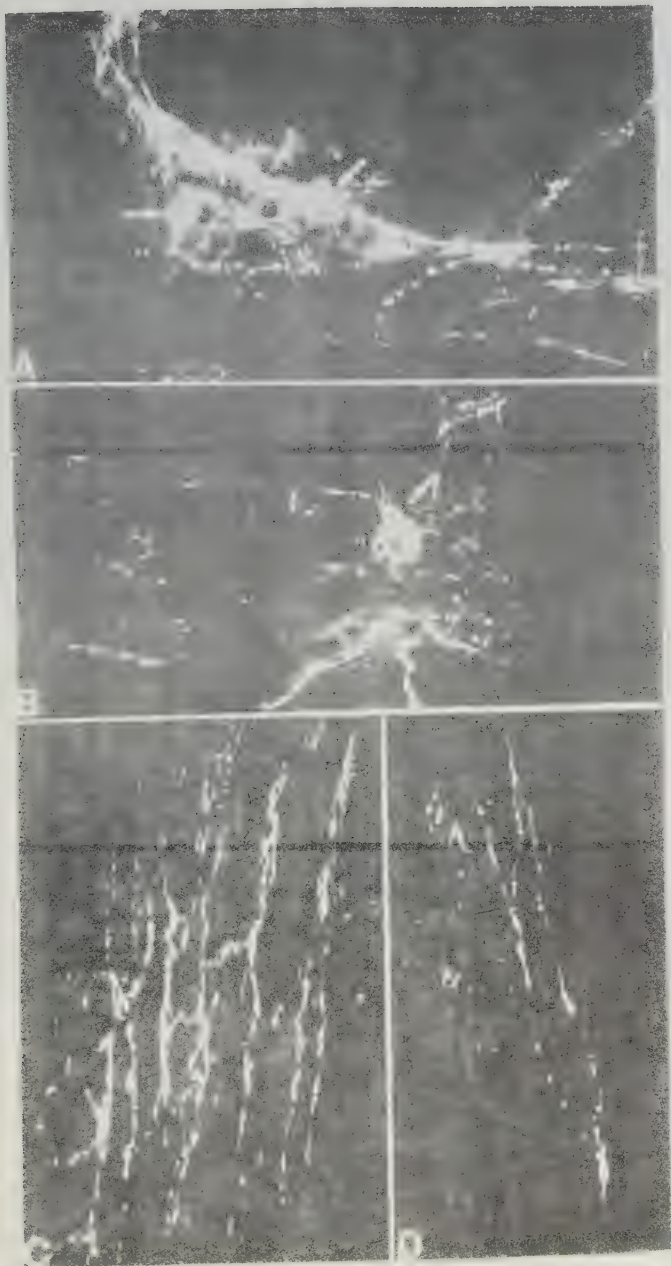
- Fig. 1. Schematic outline of the topography of peptide-containing nerve fibers and cell bodies in the oxyntic gland area of rat and mouse stomach. (Topography in pyloric gland area not different.) VIP, vasoactive intestinal polypeptide; GRP, gastrin releasing peptide; SP, substance P; CCK, cholecystokinin; NPP, neuronal type pancreatic polypeptide; SG, submucous ganglia; cm, circular muscle; MG, myenteric ganglia; lm, longitudinal muscle. For further details on the assessment see Table II.
- Fig. 2. **A.** VIP fibers in the pyloric region of the stomach of the mouse are numerous in the circular smooth muscle and in the basal part of the mucosa. m, mucosa; cm, circular muscle. **B.** and **C.** GRP fibers in the oxyntic mucosa of rat (**B**) and mouse (**C**) extend high up in between the glands. **D.** GRP-immunoreactive nerve cell body (arrow) in the myenteric ganglia in the pyloric region of a colchicine treated rat. **E.** A rich supply of SP-immunoreactive nerve fibers in the myenteric ganglia in the oxyntic gland area of the mouse. Delicate nerve fibers in the smooth muscle layers. A x 125, B and C x 150, C x 300, E x 250.
- Fig. 3. GRP-immunoreactive nerve fibers and cell bodies in whole mounts. **A.** Longitudinal muscle with myenteric ganglia in rat stomach oxyntic gland area. **B.** and **C.** Circular smooth muscle in the same location and same species. Immunoreactive fibers in bundle (**B**), and as a delicate plexus (**C**). A and C x 500, B x 250.
- Fig. 4. Whole mounts of rat stomach oxyntic gland area. **A.** Enkephalin-immunoreactive nerve fibers and cell bodies (arrows) in myenteric ganglia. Few delicate fibers in the longitudinal muscle layer. **B.** and **C.** NPY-immunoreactive (NPY antiserum) nerve fibers and cell body (arrow) in the myenteric ganglia and longitudinal muscle layer (**B**) and circular muscle layer (**C**). **D.** Gastrin/CCK-immunoreactive nerve fibers in circular smooth muscle. A and B x 350, C and D x 300.

1. VIP
2. GRP
3. SP
4. Enkephalin
5. Somatostatin
6. Gastrin/CCK
7. NPY









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